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**Influence of Drought Stress after Anthesis on Growth and  
Yield of Wheat Cultivars from Ethiopia**

**By**

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## Declaration

I declare that the thesis hereby submitted by me for the degree of Master of Science in Agriculture (Agronomy) at the University of the Free State, South Africa, is my own independent work and has not previously been submitted by me at another University/ Faculty. I furthermore cede copyright of the thesis in favour of the University of the Free State.



Dereje Biruk Gebremedhin

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## Chapter 1

### Introduction and rationale for the study

With the current rate of population growth of the world, despite the advances made in science and technology, feeding the human population remains a considerable global challenge. According to the Food and Agriculture Organisation's (FAO) report on the state of food insecurity in the world in 1999, as based on recent estimates (1995/97), 790 million people in the developing world do not have enough to eat, (FAO, 2000). The majority of these people, undoubtedly, are living in the least developed countries, among which sub-Saharan African countries constitute a significant share.

Despite being the place of origin for many important crops that are widely distributed all over the world, Ethiopia paradoxically remains one of the countries severely affected by recurrent drought. Important crops such as Barley (*Hordeum vulgare*), Sorghum (*Sorghum bicolor*), Lentil (*Lens esculenta*), Vetch (*Lathyrus sativa*), Linseed (*Linum usitatissimum*), Safflower (*Carthamus tinctorius*) and Castor bean (*Ricinus communis*) have their centre of origin in Ethiopia (Singh, 1983).

Among the different cereal crops that grow in Ethiopia, wheat stands among the few that constitute the bulk of the annual crop production. Bread wheat (*Triticum aestivum* .L), occupying about 0.879 million hectares, is one of the five most important food crops grown in Ethiopia (FAO, 1998). According to the same source, in Africa, Ethiopia stands sixth and in sub-Saharan Africa second only to South Africa, regarding both wheat area and production.

Sown on roughly 11 % of the total cropped area nationally, wheat cultivation can be as high as 45 % in those regions of Ethiopia that are most conducive to its production (Hailu *et al.*, 1991; CSA, 1994; FAO, 1994;). Despite this huge area, however, the productivity of the crop per unit area of land is relatively low compared to that of other countries. Based on three years average data (1996 – 1998), the national average wheat yield of  $1.3 \text{ t ha}^{-1}$  is 32% below the average yields for Africa (FAO, 1998).

Of the total area used for wheat production in Ethiopia, durum wheat (tetraploid) covers 60% while bread wheat (hexaploid) covers 40% (Hailu *et al.*, 1991). Wheat has a very wide distribution and is grown in areas with sufficient rainfall as well as in drought prone areas. The wheat growing environments can be classified into two major types: Highland cool areas, >1500 m above sea level (MASL), and low altitude warm dry areas <700 MASL (Hailu *et al.*, 1991).

As is true for any crop, wheat production in Ethiopia is subject to a range of constraints that limit its productivity and production. In the drought prone areas, the most important limiting factor for crop production is the low water availability. The rainfall particularly in dry areas typically exhibits a pattern of beginning late and terminating early in the rainy season. The delay in the first rain of the season usually gives farmers some chance to change to other late season crops, but the early cessation of the rain, particularly at a late stage of crop growth, leaves hardly any options. It is this early cessation of the rain that usually causes drought conditions that strike the crops at and after anthesis, resulting in very low yields and often even in total yield failures.

This kind of rainfall pattern is also typical of arid and semi-arid areas of different parts of the world. According to Arnon (1975), while cases of crop desiccation and complete yield loss are not rare in such regions, the crops suffer periods of stress of varying duration and severity. Ritchie (1980) stated that the major cause of the year-to-year variation in

crop yields is the fluctuation in weather conditions of which the variability in the distribution and amount of precipitation is the most important component. This condition causes uncertainty to the farmer regarding the soil water content at planting. The degree of uncertainty in turn, exaggerated by the lack of knowledge of the farmer regarding the soil water holding capacity and crop water requirements.

In light of the Ethiopian situation, specifically pertaining to the drought stress condition commonly encountered during the post anthesis growth phase, this study was undertaken on wheat. Two bread wheat cultivars, namely HAR-2508, a recently developed cultivar for drought prone areas and under consideration for release, and ET-13, perceived as more drought sensitive and currently under wide use, were included in this study.

This comparative study was undertaken:

- to evaluate the relative performances of the two Ethiopian bread wheat cultivars under conditions of drought stress imposed after anthesis and
- to evaluate the effect of ComCat (a biocatalyst of plant origin) on the performances of the cultivars during the pre-anthesis and post-anthesis growth stages as well as its possible circumvention effects of the stress.

The influence of cultivar difference and ComCat treatment at different growth stages, on the vegetative growth of plants during the preanthesis growth phase in both cultivars is covered in Chapter 4. This chapter also incorporates the investigation of the influence of post anthesis water deficit stress treatment on the vegetative growth and early reproductive development of the two cultivars. Similarly, the influences of cultivar differences, water, as well as ComCat treatments on vegetative growth and yield and

yield components at harvest are dealt with in Chapter 5. The protein and starch contents of seed of the two cultivars as influenced by the water and ComCat treatments are covered in Chapter 6.

## Chapter 2

### Literature review

#### 2.1 Introduction

Food and fibre production is the ultimate goal of all agricultural activities and these activities have always been subjected to the influences of natural and anthropogenic conditions. Of such conditions, climatic factors are known to influence all aspects and stages of plant growth and hence agricultural productivity, as well as the stability of production. The influence extends from the upper reaches of the atmosphere, in which spores and pollen are encountered, to the soil depth penetrated by the roots (Arnon, 1992). Moreover, all forms of life on earth owe their existence, directly or indirectly, to the availability of water. Particularly in the realm of agriculture, water is a completely indispensable necessity.

Drought has been described by the World Meteorological Organization as having a subtle beginning, an insidious progress and a devastating effect (WMO, 1975). Looking back through recorded history, more often than not, drought has been a problem for mankind. Its direct and indirect impacts had been the cause of loss of life of millions of people, through famine, as well as of millions of domestic and wild animals. Not only does it affect the social and economic lives of millions of people every year but also, from time to time, endangers the existence of whole nations (WMO, 1975). Thus, humanity has been and is still faced with the tremendous challenge of feeding itself under the threat of drought.

The term 'drought' has also been described in different contexts by different authors. Slavic (1975) described drought as an internal water deficit condition induced by external stresses such as soil water stress and/or atmospheric stress. Kramer (1980) described drought as an environmental stress condition of sufficient duration

to produce a plant water deficit, which in turn causes disturbances in physiological processes. According to Morgan (1984), internal water deficit in a plant is the result of both reduction in the soil water potential, which usually occurs progressively over a period of time, and fluctuation in the evaporation rate that occurs with daily changes in net radiation and humidity. The development of internal water deficit initiates a cascade of events, which arise from the vital functions of water in the life processes of plants. The previous descriptions of drought are based on the internal water status of the plant.

However, 'drought' has also been described, in meteorological terms as a phenomenon present when precipitation during a certain period is significantly than the average, or is lower than a critical value of precipitation that defines the initiation of drought (May and Milthorpe, 1962; Cunha *et.al.*, 1983; Hale and Orcutt, 1987).

## **2.2 Role of water in plants**

Water plays a critical role in plants due to its multiple functions. It is a major constituent of tissues, is a reagent in photosynthetic and hydrolytic processes, a solvent supplying the mode of translocation for metabolites and nutrients within the plant and is critically essential for cell enlargement (Larson 1975; Mengel and Kirkby 1987). It is so important that it is not incorrect to state that no metabolic or physiological processes in plants remain unaffected by a given magnitude of water deficit stress, either directly or indirectly.

### 2.3 Development of internal water deficit stress in plants

Due to the vital role of water, it is common to observe various physiological, morphological and metabolic changes place in plants when they are subject to water stress. According to Hale and Orcutt (1987), whenever the rate of water loss through transpiration exceeds the rate of water absorption by roots, the water in the conducting tissue is subject to tension (negative pressure). This results in the reduction of the water potential ( $\psi$ ) which in turn disturbs the  $\psi$  equilibrium between roots and the soil as well as among the various tissues and organs of the plant. As a result of removal of water from the soil through evapotranspiration and/or drainage, interruption of the continuity of the liquid water in the soil takes place. Following this interruption, some water remains on the surface of soil particles and some changes into vapour in the soil pores. As more water is removed from the soil, that which remains is more tightly bound to the soil particles. The  $\psi$  of the soil decreases until it eventually reaches a point at which a plant root is no longer able to remove sufficient water from soil particles to overcome the imposed deficit. This situation, if sustained for a long enough period, ultimately leads to permanent wilting of the plant.

Internal water deficit can occur not only under conditions of low soil moisture. According to Reid *et al.* (1991), factors such as inhibition of water absorption due to low soil temperature, excess salt in the soil solution, deficient soil aeration, injury to the root system, formation of vapour-filled air bubbles and embolisms in the xylem can all lead to the development of an internal water deficit in the plant. Crop plants, by virtue of their inherent genetic capabilities, respond to water deficit through various physiological, morphological and metabolic mechanisms, thereby increasing their ability to resist or tolerate the damaging effects of the stress.

## 2.4 Drought resistance in crop plants

Crop plants are constantly exposed to variations in environmental conditions and are continuously forced to develop mechanisms to adapt to the changing environment (Saini and Westgate, 2000). Several authors classified plants in terms of their drought tolerance mechanisms (May and Milthorpe, 1962; Levit, 1972; Larson, 1975; Hale and Orcutt, 1987; and Boyer, 1996). According to Boyer (1996), plants showing improved growth despite limited water supply are considered drought tolerant. Some species can avoid drought by maturing rapidly before the onset of dry conditions or by reproducing only after rain. Others can postpone dehydration by developing a deep root system, by protecting themselves against excessive water loss through transpiration or by accumulating large volumes of water in fleshy tissues. Some plant species, mainly lower order organisms and seed of angiosperms, allow dehydration of the tissue and simply tolerate drought conditions by continuing to grow when dehydrated or by surviving severe desiccation.

Metabolic adjustments towards tolerance are also known to take place in plants that are exposed to different kinds of biotic and abiotic stresses. One such adjustment involves the initiation of antioxidative defense mechanisms. Ingram and Bartels (1996) reported on the occurrence of increased levels of free radicals in plants during drought stress. These authors presented substantiating evidence for genes to encode for specific enzymes that detoxify active free radical oxygen species, such as ascorbate peroxidase and superoxide dismutase. These enzymes are upregulated in response to drought. Similarly, Munne-Bosch *et al.* (1999) noted a 15-fold increase in the concentration of  $\alpha$ -tocopherol (Vit.E) and a 26 % increase in carotenoids in field grown rosemary plants subjected to drought. The authors further commented on the contribution of these compounds in the prevention of oxidative damage in plants exposed to drought. Boyer (1996) also reported on the destructive property of oxygen containing free radicals on membrane components, particularly on chloroplast membranes.

Loggini *et al.* (1999) found antioxidative defense mechanisms to be responsible for the variation observed in the photosynthetic efficiency of two cultivars of wheat (*Triticum durum*) that differ in their relative sensitivity to water deficit stress. Upon dehydration a decline in chlorophyll *a*, lutein, neoxanthin and carotene contents and an increase in the pool of de-epoxidized xanthophyll-cycle components (zeaxanthin and antheraxanthin) were evident in the more sensitive cultivar. The authors further reported that, due to these observed effects, the sensitive cultivar showed a larger reduction in actual photosystem II photochemical efficiency and an increase in non-radiative energy dissipation than in the relatively-more drought resistant cultivar, after exposure to drought.

Another mechanism used by many plants, including wheat, to tolerate water deficit stress is associated with osmoregulation. According to McDonald and Davies (1996), effective osmotic adjustment in aerial plant parts can result in a yield advantage because turgor maintenance can be equated with growth maintenance at a low water potential. Similarly, Morgan (1983) reported that wheat genotypes with high osmotic adjustment capability had 1-60 % higher yields than those with low osmotic adjustment capability when water supply was limited. Blum *et al.* (1999) reported on the existence of genetic differences among wheat cultivars with high or low osmotic adjustment capability and that cultivars with high osmotic adjustment capability tended to yield better than cultivars with a low capability. In addition, and based on evidence provided by a number of authors on wheat and other crops, Turner (1997) concluded that osmotic adjustment improves water use by the crop and, because it also delays senescence and maintains assimilate transfer to the grain, it increases harvest index. However, there is a limitation as to how far this adjustment benefits the plants. Hanson and Hitz (1982) reported that, although osmotic adjustment contributes to the ability of many mesophytic plants to function metabolically at low total water potential values, the contribution is more pronounced where the decline in the water status is gradual. In agreement with this, and emphasizing the limitations of the adjustment, Aspinal (1980) mentioned that the upper limit for osmotic adjustment varies within species, but can be as great as  $-2.0$

MPa. The author further mentioned that the extent to which such adjustment occurs diminishes as the intensity of water stress increases and the capacity to adjust is usually lost altogether at drying rates greater than 1 MPa per day.

Osmoregulation is achieved by accumulation of different amino acids, ions, organic acids and sugars (Hanson and Hitz, 1982; Morgan, 1984; Mengel and Kirkby, 1987; Reid *et al.*, 1991; Ingram and Bartels, 1996; Turner, 1997). Regarding the mechanism by which sugars mitigate the effects of turgor loss due to water deficit, Boyer (1996) reported that sugars having the appropriate stereostructure might form hydrogen bonds with cell membranes where water would ordinarily bind. Since the sugars would remain as water is removed, the bonding would be stable and the membrane structure might be maintained where it otherwise would become disorganized.

Munns *et al.* (1979) measured the change in the concentration of free amino acids in leaf 7 and the apex of wheat under water deficit stress conditions. They found increased concentrations of 19 free amino acids in both leaf 7 and the apex, with proline and asparagin concentrations increasing most, while more amino acids accumulated in the apex than in leaf 7. The authors emphasized that this increase in free amino acids is one strategy of survival and perpetuation growth of the wheat plant in water limited situations.

Considering reports available in the literature, increases of proline in plants during water deficit stress seems to be the highest in most cases compared to other amino acids. According to Hanson and Hitz (1982), a very marked increase (10 to 100 fold) of the free proline content occurs in leaf tissue of many mesophytic flowering plants after hours or days of moderate to severe water stress. Bogges *et al.* (1976) reported that the increase in proline in drought stressed plants is the result of *de novo* synthesis and not due to protein degradation.

Based on the evaluation of various experimental findings, Hanson and Hitz (1982) identified three metabolic causes for proline accumulation namely, stimulated

synthesis from glutamate which reflects loss of feed back control, lowered rate of proline oxidation and slowed incorporation of proline into protein.

In addition to proline, betaine is another amino acid of which increased accumulation during various stresses, including water stress is commonly observed (Bradford and Hsiao, 1982; Hale and Orcutt, 1987; Reid *et al.*, 1991; Ingram and Bartels, 1996). According to Hale and Orcutt (1987), upon wilting due to drought stress, barley leaves accumulated betaine at a rate of 200 nM 10 cm<sup>3</sup> leaf area day<sup>-1</sup>. The betaine increase, as was the case with proline, was also found to be the result of *de novo* synthesis from serine (Hanson and Hitz, 1982).

Attempts by means of genetic engineering have been made to equip those plants that naturally lack the capacity to produce betaine, with this capacity during stress. In this regard, Huang *et al.* (2000) noted a moderate stress tolerance in transgenic lines of the test plants, *Arabidopsis*, *Brassica napus* and *Nicotiana tabacum*, based on relative shoot growth. In this experiment, the metabolic step for oxidation of choline, a ubiquitous substance, was installed into the plants by constitutive expression of a bacterial choline oxidase gene.

The significance of increases in free amino acids, particularly the significant role of increases in proline levels in the survival of drought stressed plants is not interpreted the same way by all investigators. Nonetheless, different authors have shown that proline, as an osmoticum, contributes to the osmotic potential ( $\psi_{\pi}$ ) of cells (Larson, 1975; Munns *et al.*, 1979; Reid *et al.*, 1991; McDonald and Davies, 1996; Boyer, 1996).

## **2.5 Impacts of drought stress on growth and development of wheat during the vegetative phase**

Under field conditions, where rainfall is the sole source of water supply, crops are likely to experience some degree of drought stress before completing their life cycle. Nevertheless, the extent and nature of damage, the capacity for recovery and the impact on yield depends on the development stage at which the stress is encountered (Saini and Westgate, 2000).

Wheat can be affected by water deficit stress at all its ontogenic growth stages, from germination to physiological maturity. Although wheat crops are usually planted when soil water conditions are favorable, there are certain conditions where soil moisture levels may be quite low at planting or where soil salinity reduces water availability (Simpson, 1981). Under such conditions germination can be seriously affected. During the initial stages of water imbibition, when the seeds are placed in soil with a low water potential, imbibition can take place because of the extremely low osmotic potential of the dry seed. However, as imbibition proceeds and the seed's osmotic potential rises, a point is reached at which no more water is imbibed. If this point is reached before the threshold moisture content for germination is attained, the seed will not germinate.

Following germination of the wheat kernel, the subsequent stages of root and shoot growth are also influenced by water deficit stress. Entz *et al.*, (1992) working on spring and winter wheat cultivars, report that total root length and rooting depth increased with an increased level of available soil water. Simpson (1981), and references therein, also reported decreased root growth of common wheat cultivars due to dry soil conditions. The effect of water deficit on root growth and development has a direct bearing on nutrient uptake. In this regard, Bradford and Hsiao (1982) suggested that the decrease in transpiration rate and the subsequent inhibition of nutrient uptake and unloading into the xylem are the factors involved in the stress condition plants are subjected to.

McDonald and Davies (1996) reported that, in comparison, root growth is less inhibited than shoot growth under drought conditions. The authors attribute this to the possible enhanced carbohydrate supply from the shoot and the situation probably leads to a lower sink potential of the shoot than the roots. With regard to shoot growth under drought conditions, Schuppler *et al.* (1998), during their experiment on wheat seedlings subjected to mild water stress, observed a 50% reduction in the leaf elongation rate and a 42% reduction in the mitotic activity of mesophyll cells. Eastham *et al.* (1984) also reported a reduction of leaf sheath and lamina elongation in wheat plants under water deficit stress with a concomitant reduction in total radiation interception as well as the rate of photosynthesis. Several authors have reported on the long lasting effects of leaf injuries caused by water deficit and other stresses. In this regard Benbella and Paulsen (1998) provided evidence as to how longevity of leaves, which is greatly diminished by environmental stresses, directly influences wheat yields.

The effect of water deficit stress during vegetative growth of wheat as well as other cereals is directly related to vital metabolic processes, including photosynthesis. According to Bradford and Hsiao (1982), net photosynthesis is progressively reduced by drought conditions depending on the crop and the severity of the environmental conditions. Under severe drought conditions, the photosynthate content can be reduced due to continued respiration. The reduction in photosynthesis rate is mediated partly by impeded CO<sub>2</sub> supply following stomata closure (Osmond *et al.*, 1980) and partly by a direct effect of dehydration on the photosynthetic apparatus (Hale and Orcutt, 1987). According to Naqvi (1994), the effect of water deficit stress on stomatal aperture is among the early events occurring in drought stressed plants. Kozlowsky (1972) described the water potential gradient that develops between the guard cells and subsidiary cells surrounding the stomata as an important factor leading to closure of stomata during water deficit stress. The phytohormone abscisic acid (ABA) was also implicated in the past as a major factor causing closure of stomata during water deficit stress (Livine and Vaadia, 1972; Naqvi, 1994; Hartung and Davies, 1994; Turner, 1997; Leung and Giraudat, 1998).

Reduction in the photosynthetic rate during water deficit stress is also attributed to the progressive reduction in the capacity of sinks for photosynthates. Gifford and Evans (1981) reported that sinks themselves, through feed back control, in part determine the amount of photosynthetic substrate available for distribution. In agreement with this, Ho (1988) also reported that the capacity for dry matter production in leaves might either be higher or lower than the capacity of dry matter accumulation in other parts of the plant. Slafer and Savin (1994) concluded that wheat yield can either sink or source limited.

## **2.6 Impacts of drought stress on growth and development of wheat during reproductive development**

The reproductive phase of the wheat plant, similar to its vegetative phase, is an extremely important stage in terms of the impact of drought stress. The final yield of wheat is principally determined by the number, size and mass of individual kernels (Shanahan *et al.*, 1984; Coles *et al.*, 1997). Similar observations were also made by Giunta *et al.* (1993), where severe water stress caused a huge reduction in all yield components of wheat, particularly in the number of fertile spikes per unit area (-60%) and in the number of grains per spike (-48%). The authors further reported that, under mild water stress conditions, reduction in yield was solely due to a lower grain mass. These reports confirmed that water deficit stress, occurring at that stage of development when kernel number and size are determined, had negative effects on the final yield.

Saini and Westgate (2000) divided the reproductive phase of grain crops, which is known to start with the transformation of a vegetative meristem into an inflorescence and flower primordia and ends when the seeds reach physiological maturity, into a number of sub-stages. These include floral initiation, differentiation of various parts of an inflorescence (and/or flower), male and female meiosis, development of pollen

and embryo sac, pollination, fertilization and seed development. They strongly emphasized that drought occurring during each of these sub-stages has quite specific effects, all of which lead to a decline in yield.

A study by Johnson and Moss (1976) has shown that, in the wheat plant, even if plants were not subjected to drought stress during the differentiation of the reproductive primordia, water deficiency in the subsequent stages of growth had serious effects on final yield, probably through effects on cell enlargement. They reported that water deficit stress during grain filling resulted in a 20% reduction in grain yield. This reduction appeared to be related to a reduction in the production and translocation of photosynthates from sites of synthesis to the developing grains.

Several authors have emphasized the important role photosynthesis played during the post anthesis period. In most cereals, photosynthesis taking place during the post anthesis phase contributes more photosynthate to the developing grain than the translocation of photosynthate reserves produced during the preanthesis period (Simpson, 1981). In agreement with this, Rawson and Evans, (1971), working on a range of wheat cultivars of different heights, found the contribution of stem reserves to be 10% at most. Turner (1997) reported that preanthesis assimilates contributed little to grain yield increases when the water supply was abundant, but could contribute up to 30% of the final grain yield when water deficit developed during grain filling. Kobata *et al.* (1992) emphasize that the contribution of preanthesis reserves to grain growth is dependent on the rate of development of the water deficit. They found that when water deficit developed rapidly, grain growth slowed down earlier, and the photosynthesis rate also decreased earlier. Under these circumstances the proportion, but not the absolute amount, of preanthesis dry matter transferred to the grain was higher compared to when the rate of development of the deficit was slow. The authors also reported that under such conditions late formed tillers, even when they were fertile and already contained grain, also transferred dry matter to the main stem and to other first formed tillers. Palta *et al.* (1994) confirmed the latter by means of stable isotopes of carbon and nitrogen. Their study showed that rapid stress development reduced post anthesis carbon assimilation by 57% but

increased the remobilization of preanthesis carbon by 36% compared to slow stress development. Gebbing and Schnyder (1999) also used labeled carbon in their study on wheat grown under normal conditions, and observed that preanthesis reserves contributed between 30 – 47% of the carbon in protein and 8 – 27% of the carbon in carbohydrates of the grains. The latter confirmed the lower contribution of preanthesis reserves to grain development. Boyer (1996) and Turner (1997), however, mentioned that although the contribution of preanthesis reserves is small, this still could make a difference as increased dry matter accumulation improves the plants' survival during terminal stress.

## 2.7 Drought stress and protein metabolism

In addition to its effects on wheat yields, water deficit stress also affects the quality of the harvested grain through different mechanisms, including physical and nutritive qualities. Protein is a primary quality component of wheat (Gauer *et al.*, 1992) and is influenced by both genotypic factors (Graybosch *et al.*, 1990; Triboli *et al.*, 2000) and environmental factors that are difficult to separate (Fowler *et al.*, 1990; Motzo *et al.*, 1996). Alteration to protein metabolism is one of the important events taking place in drought stressed plants. Normal development of the wheat kernel involves the synthesis and accumulation of principal storage components, i.e. protein, starch and lipids (Simmonds and O'Brein, 1981) in the endosperm and aleurone layers. The protein fraction of the kernel is generally divided into storage proteins and 'metabolic' proteins. The storage proteins account for the major part of the protein and their principal function is to serve as a store of nitrogen, carbon and sulphur during germination (Shewry and Miffin, 1985; Higgins, 1984). The 'metabolic' proteins include those that are utilized by the kernel itself for normal cell metabolism.

The storage protein fraction of seeds contains relatively few different protein species while the 'metabolic' fraction contains relatively numerous proteins but in small

amounts (Higgins, 1984). The protein bodies or sub-cellular structures contain the storage proteins that are hydrolyzed to amino acids by proteolytic enzymes (Ashton, 1976). Storage proteins are subdivided, based on their solubility, into four different classes namely albumins (soluble in water), globulins (soluble in dilute salt solutions), glutelins (soluble in weak acidic or basic solutions) and prolamines (soluble in 70 – 80% ethanol) (Higgins 1984; Skerritt, 1988). In most commonly grown cereals, with the exception of oats (Higgins, 1984) and rice, 40 – 60% of the storage proteins consist of prolamins and 20 – 40% of glutelins (Ashton, 1976).

The protein content of wheat grains varies from 7 – 15% of the dry mass (Shewry and Miffin, 1985) and is relatively low compared to that of leguminous seeds (Payne, 1987). The synthesis and accumulation of the principal storage components in the wheat caryopsis, and also in other cereals, is influenced by genetically and environmentally determined factors, the combined effect of which place an upper limit on protein content, grain mass and yield (Simmonds and O'Brein, 1981). Donovan *et al.* (1977), working on two different cultivars of wheat differing in their protein content, attributed the difference in protein synthesis capacity to differences in RNA levels. The authors reported that the high protein cultivar happened to have higher RNA levels and a higher ribosome population, both of which are associated with a higher rate of conversion of amino acids into proteins.

Under moderate stress conditions, some metabolic enzyme levels can increase, e.g. those involved in hydrolysis and dehydrogenation (Mengel and Kirkby, 1987). According to the authors, water deficit stress can also result in a decrease in the level of certain enzymes, e.g. nitrate reductase. In this regard an initial reversible inhibition of protein synthesis in young tissue, often associated with polysome disassembly and which is provoked by rapidly imposed drought stress, was reported by Bewley and Larson (1980). During prolonged stress, uptake of soil  $\text{NO}_3^-$  may be severely depressed in which case breakdown of protein in mature organs takes place. According to Hanson and Hitz (1982), the later mitigates nitrogen deficiency in young growing tissue.

In general, reports have shown an inverse relationship between moisture supply and protein content. Grant *et al.* (1991) working on barley cultivars and Gauer *et al.* (1992) as well as Rharrabti *et al.* (2000) working on wheat found a decrease in protein content at a low moisture supply and an increase in protein yield as the moisture supply increased.

## 2.8 Drought stress and carbohydrate metabolism

The metabolism of carbohydrates is also one of the important processes affected by water deficit stress during the development of the wheat kernel. Starch is the major form of carbohydrate stored in the mature wheat seed while the manner and extent of its synthesis are major determinants of grain yield (Simmonds and O'Brein, 1981). Its synthesis and accumulation in grains are greatly influenced by environmental conditions, particularly during post anthesis grain filling (Baruch *et al.*, 1979; Panozzo and Eagles, 1998).

Starch in the wheat grain comprises 65 to 70% of its dry mass (Morrel *et al.*, 1995). It consists of two types of carbohydrate polymers, amylose and amylopectin, and it occurs in predominantly larger A-type and smaller B-type granules. The relative ratio of these two types is influenced by genotype and environmental factors and this ratio is thought to have an influence on the gelatinizing and pasting properties of the kernels (Panozzo and Eagles, 1998).

Sucrose is usually the major carbon source for starch synthesis in most plants and is also the form in which carbohydrate is translocated to developing and reserve tissues from the leaves, where it is formed (Preiss, 1982). Donovan *et al.* (1977) reported that the onset of rapid starch synthesis is accompanied by a marked decline in the concentration of sucrose and reducing sugars.

Experimental evidence confirmed that, under normal conditions, the weight of starch per endosperm in the wheat grain increases rapidly and almost linearly for about 12 days after anthesis and reaches a maximum percentage of the dry weight of the endosperm about 35 days after anthesis. The later is about the point of physiological maturity (Simmonds and O'Brein, 1981). Donovan *et al.* (1977) reported that in wheat cultivars that were tested under similar environmental conditions, differences in the rate of starch accumulation among cultivars appeared to be small, but of much greater importance was the length of the time during which starch synthesis took place. This indicated that seasonal conditions such as temperature and rainfall are important factors controlling the rate and duration of starch synthesis and hence overall yield.

Under drought stress conditions there is generally an increase in sucrose content and a decrease in starch levels. This reduction in starch levels is attributed to factors such as reduced photosynthesis, increased hydrolysis of existing starch as well as a decreased rate of synthesis (Slatyer, 1969). Saari *et al.* (1985) working on wheat, rye and triticale, also reported the importance of precursor sugars in determining the final starch content.

## **2.9 Drought stress and phytohormones**

The response of crops to drought stress is known to involve phytohormones and according to Livine and Vaadia (1972), it is reasonable to assume that hormonal regulation is involved in metabolism during water deficits. Abiotic stresses impact on the hormonal system of plants and in some cases alter the levels of specific hormones or the plant's sensitivity to them (Morgan, 1990). The roles of these hormones vary depending on the type and severity of the stress, the plant type and on other environmental factors. In a number of plant species, marked and often rapid changes in hormonal levels are commonly observed in response to water deficit

stress. The inhibition of expansive growth and stomatal closure appear to be the earliest detectable responses (Naqvi, 1994).

The role of hormones in regulatory activities can be attributed to a single effect of a given hormone or to the interacting effect of different hormones. In this regard, research evidence strongly suggests that stomatal behavior is regulated by interaction between ABA, cytokinins and auxin (Naqvi, 1994). Hale and Orcutt (1987) also mentioned that virtually all known phytohormones are affected by water deficit stress and substantial evidence exists to indicate that three of the five hormones (i.e. ABA, cytokinins, and ethylene) are more involved during water stress conditions than the remaining two (i.e. indolacetic acid and gibberelin). According to the authors, water deficit stress causes an initial increase in ethylene concentration followed by a considerable increase in the concentration of ABA and a reduction in cytokinin concentration. The increase in the ABA concentration prevents further build up of ethylene while cytokinin, which stimulates ethylene synthesis, also decreases in concentration. The latter contributes further to the lowering of the ethylene concentration (Bradford and Hsiao, 1982).

Of the various hormones studied in relation to water deficit, ABA seems to stand in the forefront. It plays a major role in the adaptation of plants to abiotic environmental stresses (Zeevart and Creelman, 1988; Davies and Zhang, 1991; Naqvi, 1994; Leung and Giraudat, 1998). It is also well documented that water deficit stress causes an increase in the ABA level in a number of higher plant species (Bradford and Hsiao, 1982; Ingram and Bartels, 1996; Boyer, 1996). Although the increase in ABA concentration occurs in all organs, it primarily occurs in leaves. The increase can be the result of release from bound forms, an increase in the rate of synthesis, a decrease in the rate of destruction or a decrease in the rate of movement out of the tissue (Hale and Orcutt, 1987).

Schultz (1986) discussed the difference observed in the fate of ABA under the influence of a suddenly imposed stress and a gradually developing one. He reported that within minutes, and perhaps as a partial consequence of turgor

changes, there are: 1) changes in the intercellular distribution of ABA in leaves, and 2) changes in water and gas exchange. McDonald and Davies (1996) suggested that the maintenance of the osmotic status of roots at a low water potential is one of the regulatory roles of ABA while loss of turgor was suggested by Bradford and Hsiao (1982) to be the trigger for the very large (up to 40-fold) increase in the ABA level during water deficit stress.

Phytohormones also play an important role sensing, amplifying and transporting signals of stresses. They are efficient in this regard as they are mobile and can therefore influence many metabolic events (Reid *et al.*, 1991). However, with regard to the monitoring of the onset of water deficit stress, there seems to be no clear and unequivocal understanding of these roles of phytohormones. The questions, as put forward by Reid *et al.* (1991) are: 1). Is there only one sensor or are there many?, 2) How is the message that water availability is limiting passed on to the rest of the plant? and 3) How does this message initiate the survival strategies of the plant? The authors stated that these questions are still far from satisfactorily answered. Moreover, they cautiously forwarded their viewpoint by stating that it seems reasonable that there will be an initial and rapidly responding sensor, such as a turgor sensor which is located in leaves.

Davies and Zhang (1991), on the other hand, reported that the leaf water status does not always play a central role in the regulation of drought responses. These authors argued that shoot growth and the physiology of plants in drying soil can be modified as a function of soil drying, even when shoot water relations are not perturbed. In supporting their argument the authors cited the findings of a split root experiment on apple trees, where withholding water from one half of the root reduced the rate of leaf area development by nearly 50% over a 3-week period even in the absence of any significant leaf water deficit. Morgan (1980) also referred to a similar split root experiment where it was found that increases in the ABA level in the leaf epidermis paralleled the decrease in leaf conductance, although the leaf water potential was not affected. The author further suggested that roots might sense the water potential of the soil and signal declining water availability before leaf turgor

drops. Similarly, Turner (1997) reported that leaf turgor does not always control stomatal conductance or leaf expansion in crop species. He reasoned that soil conditions can override leaf water relations in determining the physiological activity of the shoot and in the transduction of the signal, ABA is presumed to be involved.

McDonald and Davies (1996) based their argument on several reports dealing with the possible signalling role of ABA in drought stressed plants. According to them, ABA is synthesized in increased quantities in root cells as they dehydrate and is translocated by the transpiration stream to the shoot and leaves where it regulates both stomatal behaviour and growth. McDonald and Davies (1996) further distinguished two kinds of plants based on stomatal behavior during drought stress. There are plants whose stomatal behaviour is linked to the water supply from the soil rather than to the water status of the shoot. In these plants the water status of the shoot is often controlled by the stomata in such a way that the shoot water potential does not vary as the soil dries. They termed these plants isohydric, while other plants that show reduced water potential as the soil dries were termed anisohydric.

On the other hand, Bacon *et al.* (1998) found a correlation between decreasing leaf elongation rate of barley growing in dry soil and increasing xylem sap pH as the soil dries. Based on this finding the authors suggested that increased xylem sap pH acts as a drought signal to reduce the leaf elongation rate via an ABA dependent mechanism. Similarly, Ali *et al.* (1999) reported that soil drying in the top soil layers induced an increase in both xylem and bulk-leaf ABA content in wheat and this reduced stomatal conductance and leaf growth even before a measurable change in leaf water potential could be detected in drought stressed plants. However, some of the evidence put forward in considering ABA in relation to its possible role as a root-shoot messenger appear to be equivocal. Turner (1997) presented contradicting evidence towards ABA being the root-to-shoot message as summarized in the following two statements:

- stomatal conductance and photosynthesis are frequently maintained high until two thirds of the available water in the root zone is depleted and

- the ABA concentration in the xylem sap does not always correlate well with leaf conductance, indicating the probable existence of other inhibitors of stomatal conductance in the xylem sap.

Turner (1997) considering the evidence for and against ABA's role in this regard, nevertheless concluded that, ABA seems to be the major hormone that senses soil water depletion and, hence, provide the feed-forward root-to-shoot signal of soil conditions. The consensus therefore, is that ABA is the strongest candidate as the operative phytohormone. He also suggested that further research is required to determine the site of action of ABA in the guard cells and the variation of sensitivity of cells to its presence.

The above arguments emphasize the complexities of interactions involving hormones, in the response of plants to stress conditions. In order to understand the basic processes behind these interactions, the actions of hormones during stress must be evaluated, at the whole plant level, so that the main and interaction effects of various plant variables can be evaluated. Moreover, according to Morgan (1990) the possible role of hormones alone are simply one part of a larger and more complex biochemical and genetic mechanism operative under the influence of a stress condition such as drought. He suggested the existence of a complex regulatory system which involves multiple hormones, synthetic and breakdown pathways as well as transport mechanisms. He also admits that the role of other agents such as second messengers, target cells, receptors and biochemical actions involved are only definable at the molecular level and that a lot of future research is necessary before a clear picture will emanate.

In this study the agronomic characteristics of two Ethiopian bread wheat cultivars subject to post anthesis water deficit stress, were followed using both growth and yield component parameters. Additionally, wheat plants were treated with ComCat, a new phytohormone containing biocatalyst of plant origin, in order to monitor the possible circumvention of post anthesis drought stress effects by the exogenously supplied product.

## Chapter 3

### Materials and Methods

#### 3.1 Materials

##### 3.1.1 Plant materials:

Fresh seed of two Ethiopian bread wheat cultivars, HAR-2508 (a recently developed cultivar being tested for release) and ET-13 (which is under extensive use in Ethiopia), were used in the study.

##### 3.1.2 Chemicals:

ComCat, a biocatalyst from plant origin was used to investigate its possible role in circumventing drought stress effects on growth, development and yield components of the wheat cultivars. ComCat is a natural plant extract that contains a combination of natural substances, which are collectively involved in the regulation of plant growth and development (Agraforum, Germany - personal communication). The product promotes natural plant growth and development, physiologically assists and strengthens the plants' natural resistance mechanisms to pathogen attacks and encourages root growth. The compound is a recently developed one and is being tested under different environmental and agronomic settings under the auspices of the University of the Free State, South Africa.

A range of laboratory chemicals were purchased from Merck (Germany) for use in water-soluble protein and starch assays based on standard procedures, and were of the highest purity available.

### **3.1.3 Soil**

A light red loamy sand soil with a composition of 14% clay, 81% sand, 0.2% organic carbon, 13.4 ppm nitrogen, 9.6 ppm phosphorous, 196 ppm potassium, 560 ppm calcium, 106 ppm magnesium, and 7 ppm sodium, was used. The soil had a pH in water of 5.9. In each pot 3.30 kg of soil was used to grow the plants.

### **3.1.4 Fertilizers**

Potassium phosphate and Ammonium nitrate were used as sources of phosphorous and nitrogen respectively, (see 3.2.1.2).

## **3.2 Methods**

### **3.2.1 Crop management**

#### **3.2.1.1 Seeding**

Each pot was sown with five to six seeds of either of each of the two wheat cultivars. Thinning was carried out five days after germination and the three most healthy plants were left to grow in each pot. Early thinning was done in order to avoid the possible future complication that might have arisen during the measurement of root mass due to the root of the thinned out plants that could have remained in the pots.

#### **3.2.1.2 Soil fertilization**

Based on the laboratory analysis of nutrient content of the soil, potassium phosphate (used as source of phosphorus) was thoroughly mixed with the soil before sowing at a rate of 12 mg phosphorous per kg of soil. Nitrogen was applied in a split application. The necessary amount of ammonium nitrate was dissolved in water and supplied to each pot at a rate of 70 mg per kg of soil. The first application was done 7 days after emergence. The split applications were done three times at about equal intervals up till anthesis.

### 3.2.2 Treatments

The study involved the investigation of the interaction and individual effects of the following three main factors:

#### 3.2.2.1 Cultivar effects

Two Ethiopian bread wheat cultivars, HAR-2508 and ET-13, were used in the study to investigate their responses to the treatments they were exposed to. Comparison of the responses, as monitored by different growth and yield component parameters were used to characterize the different cultivars, as well as to establish the possible cultivar effects on the influence of an imposed post anthesis water deficit stress (water stress) condition. The latter was established by means of a standard statistical analysis procedure (see 3.2.4).

#### 3.2.2.2 Treatment with ComCat

The influence of treatment with ComCat on growth and development of the wheat cultivars was investigated, using different agronomic parameters. ComCat was used at the following four levels: 1) Control (no application of ComCat, CC-0), 2) ComCat application at growth stage 13 (CC-13), 3) ComCat application at growth stage 19 (CC-19) and 4) A double application of ComCat at growth stages 13 and 19 (CC-13 & 19). Growth stages are based on the description of Zadoks *et al.*, 1974.

In all treatments, ComCat was applied at a rate equivalent to 100 g ha<sup>-1</sup>, which is the recommended concentration (Agrorum, Germany - personal communication). The foliar application of ComCat was done in a spraying room fitted with a specially designed automatic spraying machine. The machine sprays over a length of 4 meters. The potted plants were arranged under the nozzles in such a way that each pot received the required dosage. The height, pressure and speed of the spraying

operation was carefully calibrated according to a volume equaling  $300 \text{ l ha}^{-1}$ , using water, until the desired spray volume over a row length of 4 metres was achieved.

### 3.2.2.3 Normal watering and water deficit stress treatments

The two wheat cultivars were subjected to the following two levels of water treatment:

1) 60 % to 100% of available water content (AWC) was maintained on the non-water stressed control plants throughout until the physiological maturity stage was reached, and 2) the above level of water treatment (60 – 100%) was maintained until anthesis (i.e. until growth stage 60) after which the water content was decreased to a level where only about 10% of the AWC remained, and until the physiological maturity stage was reached in water deficit stressed plants. Subsequently, the plants were re-watered to the original level of 100% AWC.

Both levels of water treatment were maintained using a gravimetric method of water content determination, (McIntyre, 1974; Labuschagne, 1989; Lowery *et al.*, 1996). Based on laboratory analysis, the field capacity of the soil was determined to be 420 ml of water per pot and the AWC was found to be 244 ml of water per pot. The non-water-stressed plants were re-watered to 100 % AWC whenever they lost about 100 ml of water while the water-stressed plants were re-watered to 100% AWC whenever they lost about 220 ml of water. In this way, the plants were kept at the desired level of water content.

In order to maintain the two levels of water content during the vegetative stage, the volume of water supplied to the plants was corrected by calculating the average of the mass gains of the two cultivars. The latter was regularly determined whenever plants were harvested for data recording at different stages. The two cultivars, therefore, received the same volume of water during the initial period of growth, i.e. until the second set of data was collected at growth stage 19. Until this stage, no significant growth differences between cultivars were observed. However, following the measurement of the mass of the plants at growth stage 19, the plants from different

cultivars showed considerable variation in their mass. The watering was, therefore, adjusted to this mass separately for the two cultivars (as described above).

### **3.2.3 Experimental design and data measurement**

#### **3.2.3.1 Experimental design**

The greenhouse study involved two separate experiments, which were started at the same time and in the same greenhouse, using factorial randomized complete block designs (RCBD) with three blocks. Experiment 1 was designed to monitor the vegetative growth pattern of wheat, until the early stages of grain filling, as influenced by cultivar, ComCat and post anthesis water deficit stress. Four different sets of data were collected using different growth parameters, at growth stages 13, 19, 34, and 73-77. As the ComCat and water stress treatments were administered at different growth stages, the number of treatment (or factor) combinations that were monitored at each of the four growth stages was different. The details for each growth stage are described as follows:

#### **Growth stage 13**

Until plants reached growth stage 13, the only factor or variable considered was cultivar difference (two cultivars). Therefore, a 1 X 2 factorial RCBD was used (Table 3.1).

**Table 3.1:** Combinations of treatments used for the data collected at growth stage 13.

Cultivar	Treatment
HAR-2508	No chemical or water stress treatment
ET-13	No chemical or water stress treatment

**Growth stage 19**

At this stage, in addition to the possible cultivar effect, the influence of ComCat applied at growth stage 13 (see section 3.2.2.2) was investigated in comparison with the control (no ComCat applied). Therefore, a 2 X 2 factorial RCBD (four treatment combinations) was used (Table 3.2) for the parameters measured at growth stage 19.

**Table 3.2:** Combinations of treatments used for the data collected at growth stage 19.

Cultivar	Treatment
HAR-2508	No ComCat applied
HAR-2508	ComCat application at growth stage 13
ET-13	No ComCat applied
ET-13	ComCat application at growth stage 13

### Growth stage 34

This stage involved measurement of the influences of both cultivar and ComCat treatment at two and four levels respectively. Subsequently, a 2 X 4 (eight treatment combinations; Table 3.3) factorial RCBD was used for data collected at this growth stage.

**Table 3.3:** Combinations of treatments used for the data collected at growth stage 34.

Cultivar	Treatment
HAR-2508	No ComCat applied
HAR-2508	ComCat application at growth stage 13
HAR-2508	ComCat application at growth stage 19
HAR-2508	ComCat application at growth stages 13 & 19
ET-13	No ComCat applied
ET-13	ComCat application at growth stage 13
ET-13	ComCat application at growth stage 19
ET-13	ComCat application at growth stages 13 & 19

### Growth stages 73-77

At this growth stage, in addition to the two cultivar levels and the four ComCat levels, two levels of water treatment were administered. Subsequently, for data collected at this growth stage a 2 X 4 X 2 factorial RCBD (sixteen treatment combinations; Table 3.4) was used. The water stress treatment was applied at growth stage 60, which allowed the plants to be kept under water stress for a period of two weeks.

**Table 3.4:** Combinations of treatments used for data collected at growth stages 73-77.

Cultivar	Treatment
HAR-2508	No ComCat applied
HAR-2508	NWS + ComCat application at growth stage 13
HAR-2508	NWS + ComCat application at growth stage 19
HAR-2508	NWS + ComCat application at growth stages 13 & 19
-----	
ET-13	No ComCat applied
ET-13	NWS + ComCat application at growth stage 13
ET-13	NWS + ComCat application at growth stage 19
ET-13	NWS + ComCat application at growth stages 13 & 19
-----	
HAR-2508	Water stress (WS) + No ComCat applied
HAR-2508	WS + ComCat application at growth stage 13
HAR-2508	WS + ComCat application at growth stage 19
HAR-2508	WS + ComCat application at growth stages 13 & 19
-----	
ET-13	WS + No ComCat applied
ET-13	WS + ComCat application at growth stage 13
ET-13	WS + ComCat application at growth stage 19
ET-13	WS + ComCat application at growth stages 13 & 19

Experiment 2 was designed to monitor influences of post-anthesis water deficit stress, cultivar differences, and ComCat treatment on vegetative and yield components of wheat including grain protein and starch contents. The same design and treatment combinations as described above for the data collected at growth stages 73-77 were applied.

### **3.2.3.2 Data measurement**

Various parameters were used to measure growth in terms of increase in plant size or its organs, grain yield and its components as well as kernel quality in terms of protein and starch levels.

#### **3.2.3.2.1 Vegetative growth**

In investigating plant growth in terms of size of the whole plant or its organs, the following parameters were used:

- **Plant height:** measured from the base of the stem to the tip of the longest leaf (during vegetative growth) or to the tip of the spike (after anthesis), using a measuring tape.
- **Stem thickness:** measured 3 cm from the base of the main shoot during initial stages (before the stage of stem elongation), and in subsequent stages, at the base of the third node using a digital caliper. During the initial stages the base of the stem was determined as the point of junction between the primary root and the main stem.

- Leaf area : the area of freshly cut leaves was measured using a digital leaf area meter (Lambda Instruments corporation, Model LI-3000, USA).
- Root volume: roots were separated from soil by washing very gently with running tap water over a 0.5 mm<sup>2</sup> wire mesh. Clean roots were then wrapped with laboratory grade wrapping paper for half an hour to remove the excess water from the surface. The roots from a single pot were subsequently placed in a measuring cylinder, filled with a known volume of water, (250 ml) and volume of the displaced water was measured and taken as the volume of the roots (Böhm, 1979).
- Leaf dry mass: fresh leaves were placed in a paper bag and dried in an oven (Labcon, Type FSOE, West Germany) for 72 hours at 70 °C ( $\pm 2$  °C). The dry mass was subsequently measured.
- Stem dry mass: main stems and tiller stems were placed in a paper bag and dried in an oven for 72 hours at 70 °C ( $\pm 2$  °C). The dry mass was subsequently measured.
- Root dry mass : the roots were placed in a paper bag and dried in an oven for 72 hours at 70 °C, ( $\pm 2$  °C) before the dry mass was measured.

#### 3.2.3.2.2 Yield components

- Dry mass of spikes : spikes were placed in a paper bag, dried as previously described for leaves (3.2.3.2.1) and the dry mass measured.
- Number of kernels per spike: the number of kernels in the main plant and tillers were counted and divided by the number of spikes.

- Fresh air-dry mass of kernels: immediately after threshing, the fresh kernel mass from each main stem and tillers was determined.
- Total number of kernels per pot (i.e. per three plants): the kernels from both the main plant tillers in one pot were counted.
- Hundred kernel mass: immediately after threshing, all the kernels from the main plant and tillers were mixed and 100 kernels were randomly selected and weighed.
- Grain yield per square metre: the grain yield from a pot was converted to the basis of yield per square meter. The area of the pot was calculated to be 0.0214 m<sup>2</sup>.
- Harvest index: the index, which is the proportion of grain mass to total dry mass of above ground parts at harvest, was calculated according to Fisher and Turner (1978).

### **3.2.3.2.3 Determination of protein content in the harvested wheat kernels**

#### **3.2.3.2.3.1 Water soluble protein**

##### **Extraction of water soluble protein**

Water soluble protein content was extracted according to the method of Pretorius and Small (1991). The extraction buffer contained 12.5 mM TRIS (hydroxymethyl aminomethane), 2 mM EDTA (ethelene-diamine-tetra-acetic acid), 10 mM 2-mercapto-ethanol (added just before use) and 2 mM PMSF (phenylmethane sulphonyl fluoride) diluted in 1 ml ethanol. The pH was adjusted to 6.8, using a pH meter (Model 520, Orion research Incorporation, USA).

Five hundred mg (0.5 g) of fine flour was prepared by grinding the kernels using a laboratory-grade milling machine. Three replicates were weighed and ground

separately for each treatment. The flour was homogenized with the extraction buffer at a ratio of 1 g : 4 ml (i.e. 0.5 g flour: 2 ml extraction buffer) using a mortar and pestle. The homogenate was subsequently transferred to a clean Eppendorf vial, and centrifuged for ten minutes at 12 000 r.p.m. at room temperature. Ten  $\mu$ l of the supernatant was discharged in a clean Eppendorf vial and diluted 50 times with distilled water.

#### **Determination of water soluble protein content**

The protein content was determined spectrophotometrically at 595 nm by means of an ELx808 microplate reader (Biotek Instruments, USA) using the biorad method (Bradford, 1976). Bovine  $\gamma$ -globuline was used as standard and protein content was expressed as  $\text{mg g}^{-1}$  dry mass.

#### **3.2.3.2.3.2 Determination of total protein content in the harvested wheat kernels**

The total protein content was determined by measuring the total nitrogen content. This was done by Central Agricultural Laboratories (Pelindaba, South Africa). The grain nitrogen content was converted to total protein content using a factor of 6.25 (Amsal *et al.*, 1994).

### 3.2.3.2.4 Determination of starch content in the harvested wheat kernels (Boehringer Mannheim, 1997.)

#### Sample preparation

The wheat kernels were fine-ground (to pass through a 0.2 mm sieve) using a laboratory-grade milling machine. Three replicates of each treatment were weighed and ground separately. Two hundred mg (0.2 g) of ground powder was placed in a 100 ml Erlenmeyer flask and 20 ml dimethyl sulphoxide (DMSO) as well as 5 ml HCl (8 M) were added. The flask was then covered with parafilm and incubated for 30 minutes at 60 °C on a heatable magnetic stirrer (Voss Instruments Ltd., England). The solution was left to cool to room temperature, after which 50 ml distilled water was added. The pH was adjusted to 4 – 5 with NaOH (5 M) under vigorous shaking. The solution was subsequently transferred to a volumetric flask, a final volume of 100 ml obtained by adding distilled water and filtered through Whatman No 1 filter paper. The filtrate was diluted 10 times with distilled water before starch determination commenced.

#### Starch content determination

The starch content of kernels was determined enzymatically using starch test kits (Boehringer Mannheim, 1997; Cat. No. 207 748). Optical density readings were taken using an Elx808 microplate reader at 340 nm. Starch content was determined according to the calculation method of the suppliers of the test kits (Boehringer Mannheim, 1997) where starch content (C) is given by:

$$C = \frac{(V \times MW)}{\epsilon \times d \times v \times 1000} \times \Delta A \text{ (g/l)}$$

- where,
- V = final volume (ml)
  - v = sample volume (ml)
  - MW = molecular mass of starch
  - d = light path (cm)
  - $\epsilon$  = absorption coefficient of NADPH at 340 nm
  - $\Delta A$  = optical density reading of sample minus that of sample blank.

### **3.2.4 Data analysis**

Statistical computer software (MSTATC, version 2.1) was used for the analysis of data and for separation of means according to the Tukey procedure at the 5 % probability level (unless mentioned otherwise).

### **3.2.5 Greenhouse conditions**

The green house was kept at a night and day temperature of 15/23 °C, with a fluctuation of  $\pm 5$  °C.

## Chapter 4

# Vegetative growth patterns of two Ethiopian wheat cultivars, until the early stages of grain filling, as influenced by cultivar differences and ComCat as well as post anthesis water stress treatments

### 4.1 Introduction

The early stages of growth of a plant, more particularly of plants with a determinate type of growth, are known to have major effects on the late growth stages. The early vegetative growth of wheat begins after the successful germination of the mature seed. The condition of the embryo and the wheat kernel is crucially important for successful germination and for vigorous seedling development, as determined by genotype and environmental conditions (Lersten, 1987). Successful germination of a wheat seed requires a moisture level of at least 35-45% of kernel dry mass and germination can take place at temperatures between 4 and 37 °C, with 20-25 °C being optimal (Evans *et al.*, 1975).

Following germination, the seminal roots extend into the soil and the coleoptile penetrates the surface. The growing point is subsequently raised above the surface by expansion of the rhizome, i.e. the internodes above the coleoptile (Evans *et al.*, 1975). Moreover, successful seed germination and seedling emergence, as well as development of leaves and tillers, stem elongation and booting are dependent upon genotype and environmental factors. In the wheat plant, water deficit stress affects growth of roots, leaves, stems and reproductive structures as well as the length of the developmental stages (Clark *et al.*, 1981).

Since crops are grown under varying environmental conditions, the ability to adapt to stress quickly is an important aspect of their genetic potential. The response of a plant

to a stress condition, therefore, depends on its genetic potential to adapt, the duration of exposure and the growth stage at which it encounters the stress (Gusta and Chen, 1987). Among the different stresses that a growing crop plant might be subjected to in the course of its development, water deficit or drought stress of varying magnitudes and duration remains one of the most frequently encountered stress conditions. In this light, the growth performance of two Ethiopian bread wheat cultivars were investigated from the onset of early post germination growth up till early grain filling.

At different vegetative growth stages, plants were treated with ComCat. Water deficit stress was subsequently induced for a specific time interval (two weeks) directly after anthesis. Four growth stages (i.e. growth stages 13, 19, 34 and 73-77; Zadok *et. al.*, 1974) were chosen as data collection points. Details of the findings are presented for each growth stage separately, but also characterization of the growth patterns of the two cultivars through the course of the growth stages.

## **4.2 Materials and methods**

All methods applied in this chapter have been described previously (see chapter 3; sections 3.1 and 3.2).

## **4.3 Results**

### **4.3.1 Vegetative growth characteristics of two Ethiopian wheat cultivars before the application of ComCat, as measured at growth stage 13**

Up till growth stage 13, characterized by the unfolding of three leaves, the two wheat cultivars were grown under optimal glasshouse conditions and without the influence of ComCat. Therefore, only genetic or cultivar factors are to account for any differences or similarities observed.

The measurements taken at this stage indicated that the two cultivars exhibited only slight differences regarding those parameters used to measure vegetative growth in

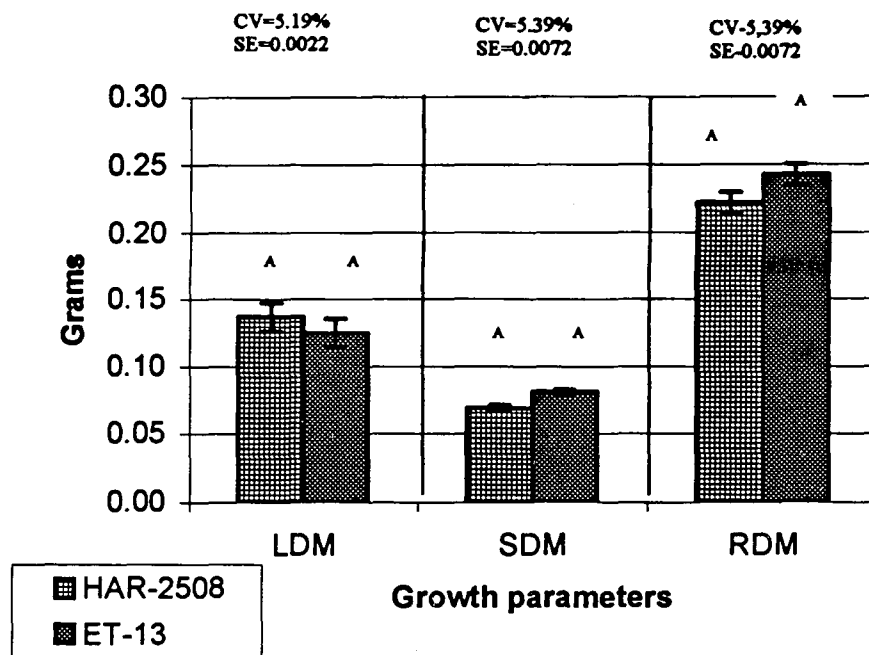
terms of size gains (i.e. plant height, stem thickness and leaf area; Table 4.1). Plants of the cultivar ET-13 were taller and had slightly thicker stems (i.e. +16% and +6% respectively) than that of plants of the cultivar HAR-2508, which in turn measured higher than the former in leaf area (i.e. +7%). The analysis of variance, however, revealed that these differences between the two cultivars were not statistically significant at the 5% probability level (Table 4.1).

**Table 4.1:** Vegetative growth variation in two Ethiopian wheat cultivars as measured at growth stage 13.

Cultivars	Plant height (cm)	Stem thickness (mm)	Leaf area (cm <sup>2</sup> )
HAR-2508	23.33 ± 2.89 A	2.02 ± 0.10 A	6.39 ± 0.48 A
ET-13	27.00 ± 2.65A	2.14 ± 0.23 A	5.98 ± 0.36 A
C.V (%)	6.49	10.66	9.14
SE	0.9428	0.1281	0.3262

Similar letters indicate non-significant difference at the 5% probability level. ± = Standard deviation for three replications. cv = Coefficient of variation. SE = Standard error.

Similarly, only slight differences were observed between the two cultivars in terms of mass gain (i.e. dry mass of leaves, stems and roots; Figure 4.1). In comparison, the cultivar HAR-2508 had higher leaf dry mass (+10%), while ET-13 measured higher in dry mass of both stems and roots(+ 17% and +9% respectively). However, as was the case with size gain., the differences observed between the two cultivars, were also not statistically significant at the 5% probability level.



**Figure 4.1:** Vegetative growth variation in two Ethiopian wheat cultivars as measured at growth stage 13. (LDM = Leaf dry mass; SDM = Stem dry mass; RDM =Root dry mass; Similar letters on top of the columns indicate non-significant differences between the two cultivars at the 5% probability level).

The lack of statistically significant differences in vegetative growth between the two cultivars, using both size and mass gain parameters, indicated that the two cultivars were growing at more or less similar rates up till growth stage 13.

#### 4.3.2 Vegetative growth characteristics of two Ethiopian wheat cultivars at growth stage 19

Unlike the preceding growth stage, the growth performances of the two wheat cultivars at growth stage 19 were influenced by foliar spray treatment with ComCat at growth stage 13. Hence, there were four treatment combinations, involving the two cultivars and the two levels of ComCat that needed to be considered.

#### **4.3.2.1 Vegetative growth variation at growth stage 19 in two Ethiopian wheat cultivars as influenced by treatment with ComCat at growth stage 13**

The interpretation of raw data obtained for each of the four treatments (average of three replications) revealed some differences in growth patterns between the cultivars in terms of size and mass gain. Application of ComCat at growth stage 13 increased mean plant height of HAR-2508 slightly (+3%) but the same treatment reduced the height of ET-13 by 14% compared to their untreated controls (Table 4.2). The same tendency was observed for stem thickness and leaf area where treatment with ComCat tended to improve the growth of these organs in HAR-2508 while reducing their growth in ET-13. However, none of these differences were statistically significant at the 5% probability level. Nevertheless, the general pattern was that ComCat treatment at growth stage 13 tended to affect the growth pattern of HAR-2508 in a positive way while inhibiting growth slightly in ET-13 between growth stages 13 and 19.

**Table 4.2:** Influence of ComCat treatment at growth stage 13 on vegetative growth of two Ethiopian wheat cultivars in terms of size as measured at growth stage 19.

Growth parameters	Cultivars and treatments				CV (%)	SE
	HAR-2508		ET-13			
	CC-0	CC-13	CC-0	CC-13		
Plant height (cm)	35.67 ± 0.50 A	36.87 ± 5.44 A	42.73 ± 5.81 A	36.63 ± 7.58 A	15.55	3.4089
Stem thickness (mm)	3.65 ± 0.11 A	3.74 ± 0.21 A	3.32 ± 0.22 A	3.18 ± 0.05 A	4.46	0.0895
Leaf area (cm <sup>2</sup> )	410.96 ± 26.21A	429.40 ± 52.15A	573.69 ± 83.92A	541.68 ± 28.29A	10.21	28.8264

Similar letters indicate non-significant differences at the 5% probability level. ± = Standard deviation for three replications.

The growth differences observed by means of size gain parameters were not observed in terms of mass gain parameters (Table 4.3). Although the dry mass of roots of HAR-2508 plants treated with ComCat at growth stage 13 (CC-13) tended to be slightly higher than that of the untreated control plants, for the rest of the parameters in HAR-2508 and all of the parameters in ET-13, the untreated control plants measured higher than the treated ones (Table 4.3). This indicated that ComCat application (at CC-13) had no effect in terms of mass gain.

**Table 4.3:** Influence of ComCat treatment at growth stage 13 on vegetative growth of two Ethiopian wheat cultivars in terms of mass gain as measured at growth stage 19.

Growth parameters	Cultivars and treatments				CV (%)	SE
	HAR-2508		ET-13			
	CC-0	CC-13	CC-0	CC-13		
Leaf dry mass (g)	1.41 ± 0.19 A	1.40 ± 0.14 A	1.55 ± 0.15 A	1.54 ± 0.20 A	13.35	0.1137
Stem dry mass (g)	0.89 ± 0.21 A	0.86 ± 0.11 A	0.98 ± 0.07 A	0.94 ± 0.05 A	14.81	0.0786
Root volume (cm <sup>3</sup> )	13.33 ± 1.29 A	12.90 ± 1.82 A	14.20 ± 2.03 A	13.17 ± 1.66 A	14.17	1.0963
Root dry mass (g)	1.62 ± 0.44 A	1.72 ± 0.19 A	1.87 ± 0.13 A	1.53 ± 0.11 A	13.54	0.1316

Similar letters indicate non-significant differences at the 5% probability level. ± = Standard deviation for three replications.

Subsequently, the above data, in terms of mass gain was subjected to statistical analysis of variance (Table 4.3) which revealed no significant interaction effect, and thus no significant differences among the treatments at the 5% probability level.

In summary, and all measured parameters considered, it appeared that the two cultivars were still growing at more or less the same rate at this growth stage, despite the application of ComCat.

#### 4.3.2.2 Statistical analysis of the correlation between different growth parameters as measured at growth stage 19

In order to identify the relationships that might have existed among the different growth parameters, a statistical correlation analysis of the parameters that were measured at

growth stage 19 was performed (values pooled over the three replications of each of the four treatments; Table 4.4). This analysis revealed a highly significant positive correlation ( $P < 0.01$ ) between leaf dry mass and stem dry mass, leaf dry mass and root volume as well as stem dry mass and root dry mass. Plant height showed a non-significant weak association with most of the parameters except with leaf dry mass and leaf area. Similarly, stem thickness had a very weak non-significant association with most of the parameters except with root volume. Stem dry mass showed a weak relationship with root volume.

**Table 4.4:** Coefficients of correlation between different growth parameters measured at growth stage 19.

	Height	Stem thickness	Leaf area	Leaf dry mass	Stem dry mass	Root volume
Stem thickness	0.02 <sup>ns</sup>					
Leaf area	0.56*	-0.42 <sup>ns</sup>				
Leaf dry mass	0.57*	-0.10 <sup>ns</sup>	0.65*			
Stem dry mass	0.42 <sup>ns</sup>	-0.05 <sup>ns</sup>	0.56*	0.81**		
Root volume	0.49 <sup>ns</sup>	0.18*	0.53 <sup>ns</sup>	0.77**	0.51 <sup>ns</sup>	
Root dry mass	0.25 <sup>ns</sup>	0.13 <sup>ns</sup>	0.16 <sup>ns</sup>	0.53 <sup>ns</sup>	0.71**	0.37 <sup>ns</sup>

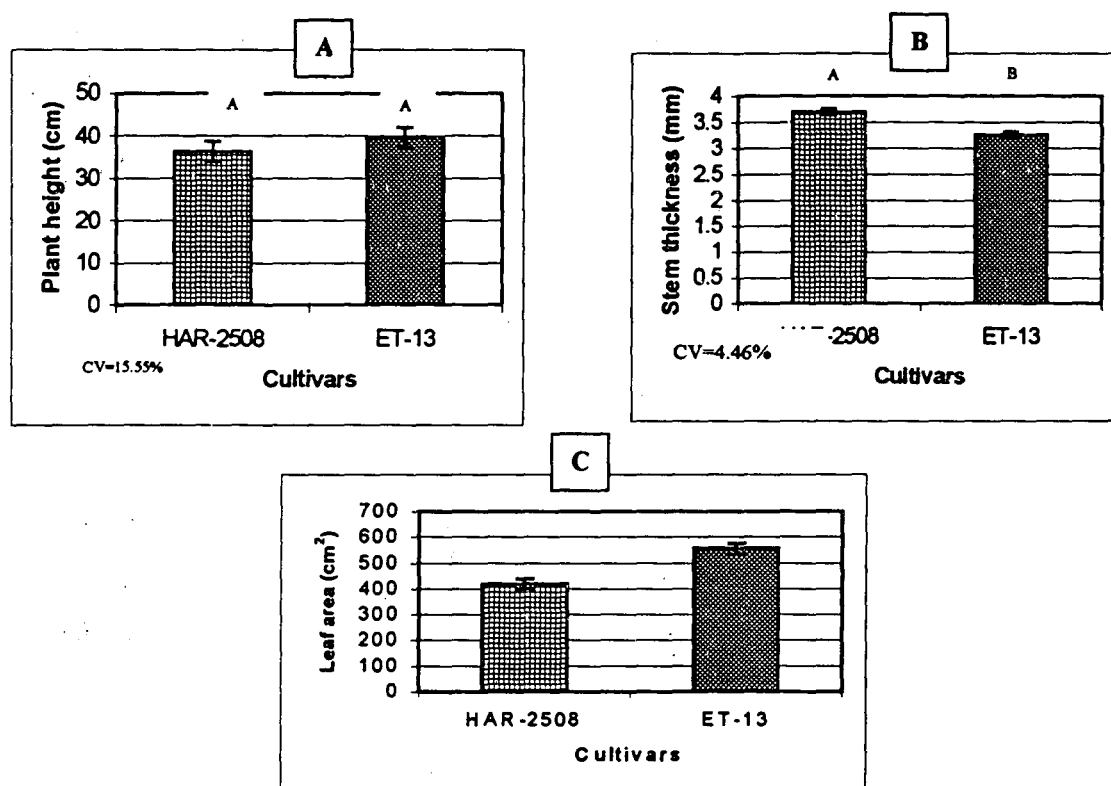
\*, \*\* = correlation significant at 5 and 1% probability levels respectively; ns = non-significant correlation at the 5% probability level.

#### 4.3.2.3 Statistical analysis of the individual effects of cultivar differences as well as ComCat treatments on the vegetative growth of two Ethiopian wheat cultivars, as measured at growth stage 19

Following the outcome of the analysis of the effects of each of the treatments on the different growth parameters in the previous section, statistical analysis of the individual effects of each of the two main factors, namely cultivar differences and ComCat treatments, on various parameters that measure size and mass gains was additionally performed.

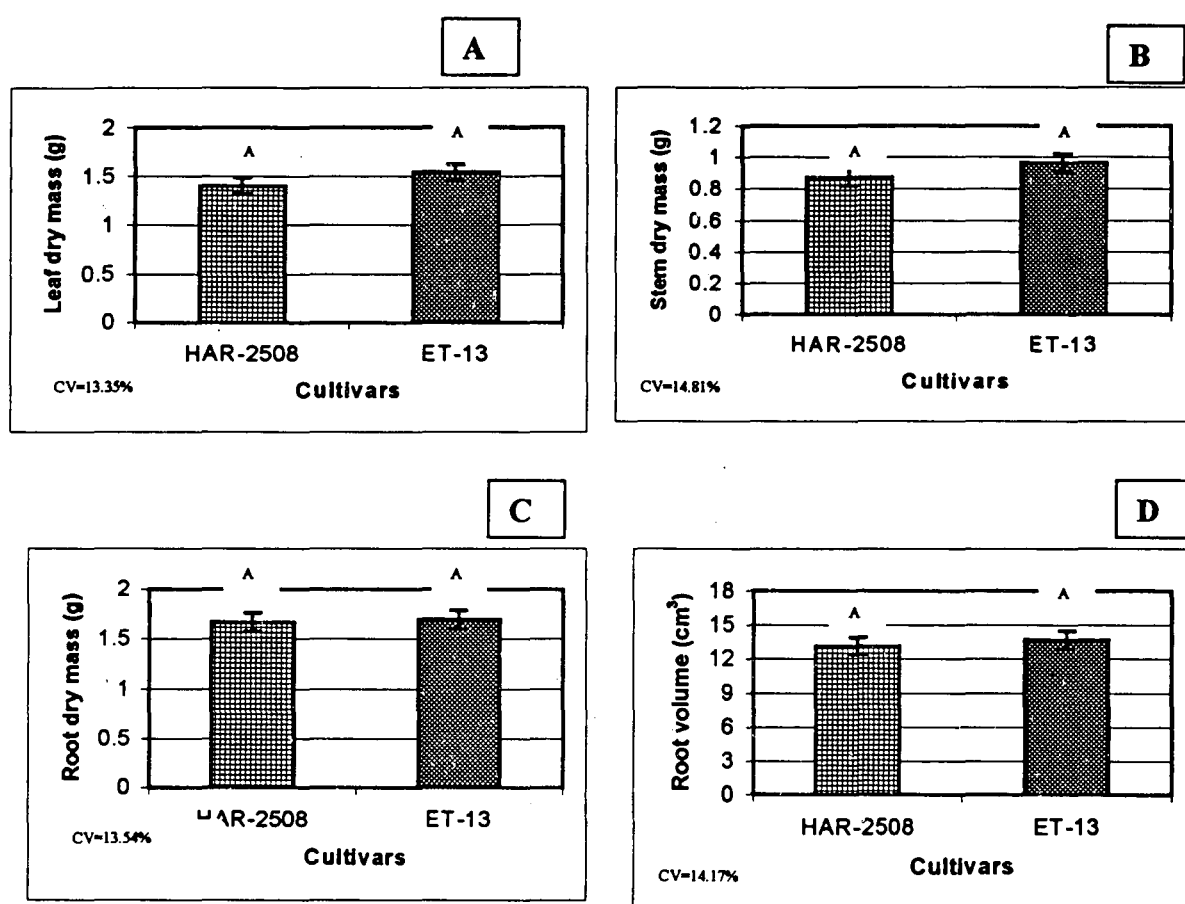
#### 4.3.2.3.1 Cultivar influence

4.3.2.3.2 With regard to plant height (values pooled for ComCat treatments), the cultivar ET-13 grew 8% taller than HAR-2508 (Figure 4.2A). This difference, however, was not statistically significant at the 5% probability level indicating similar growth rates for the two cultivars in terms of height, up till growth stage 19. On the other hand, the two cultivars differed significantly ( $p < 0.01$ ), regarding their mean stem thickness. Stems of HAR-2508 were 12% thicker than those of ET-13 (Figure 4.2B). The same significant difference between cultivars was observed for leaf area where leaves of ET-13 were 25% larger than those of HAR-2508 (Figure 4.2C).



**Figure 4.2 :** Influence of cultivar differences on vegetative growth of wheat cultivars as measured at growth stage 19 in terms of parameters that measure size gains, namely A) plant height, B) stem thickness and C) leaf area. (Different letters on top of the columns indicate significant difference between the mean values at the 5% probability level, while similar letters indicate non-significant difference).

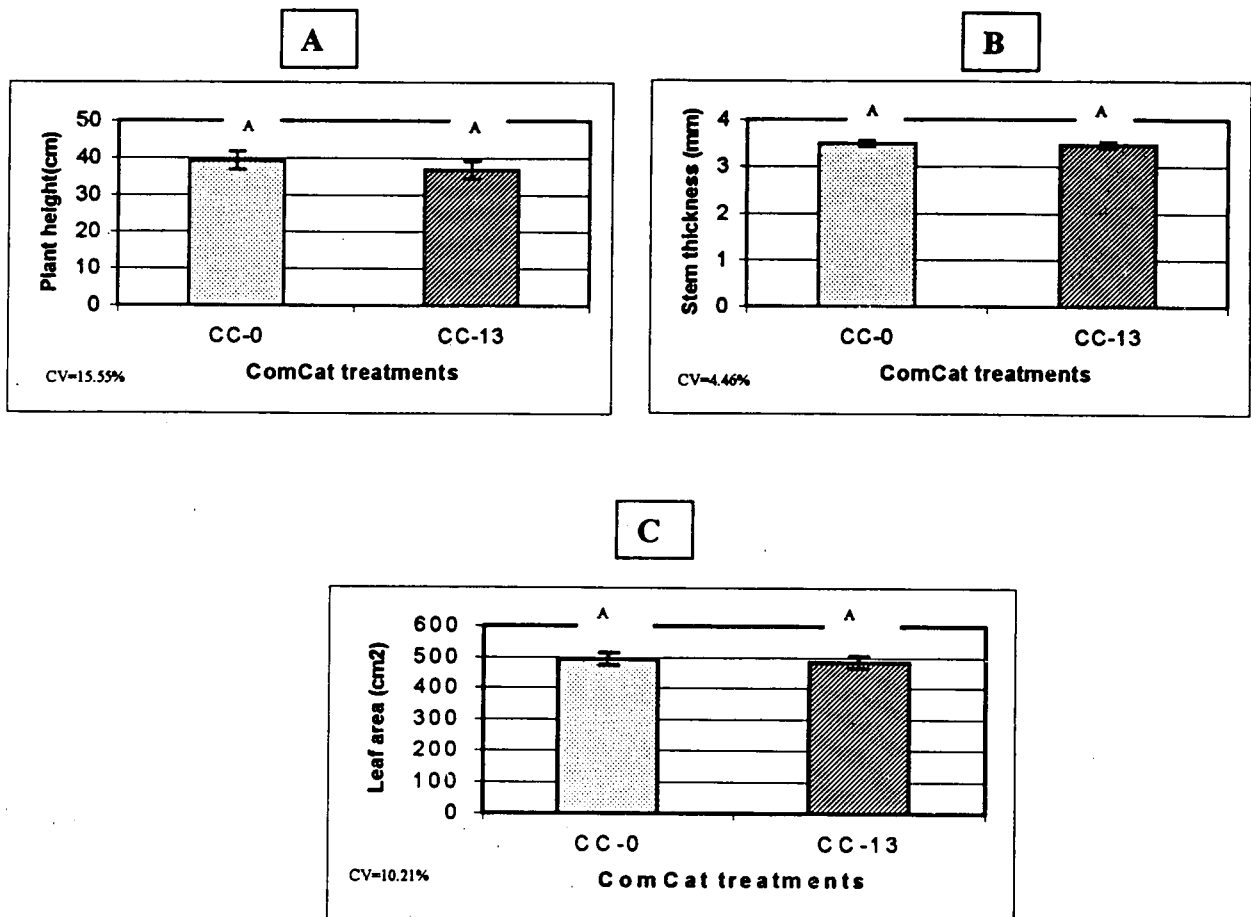
With regard to the cultivar characteristics, measured in terms of mass gain, slight differences were observed between the two cultivars (Figure 4.3A, B, C and D). The cultivar ET-13 showed slightly higher mean values of dry masses of leaves, stems and roots as well as in root volume (i.e. +10%, +10%, +2% and +4%, respectively) than HAR-2508. Statistically, these differences were, however, non-significant at the 5% probability level.



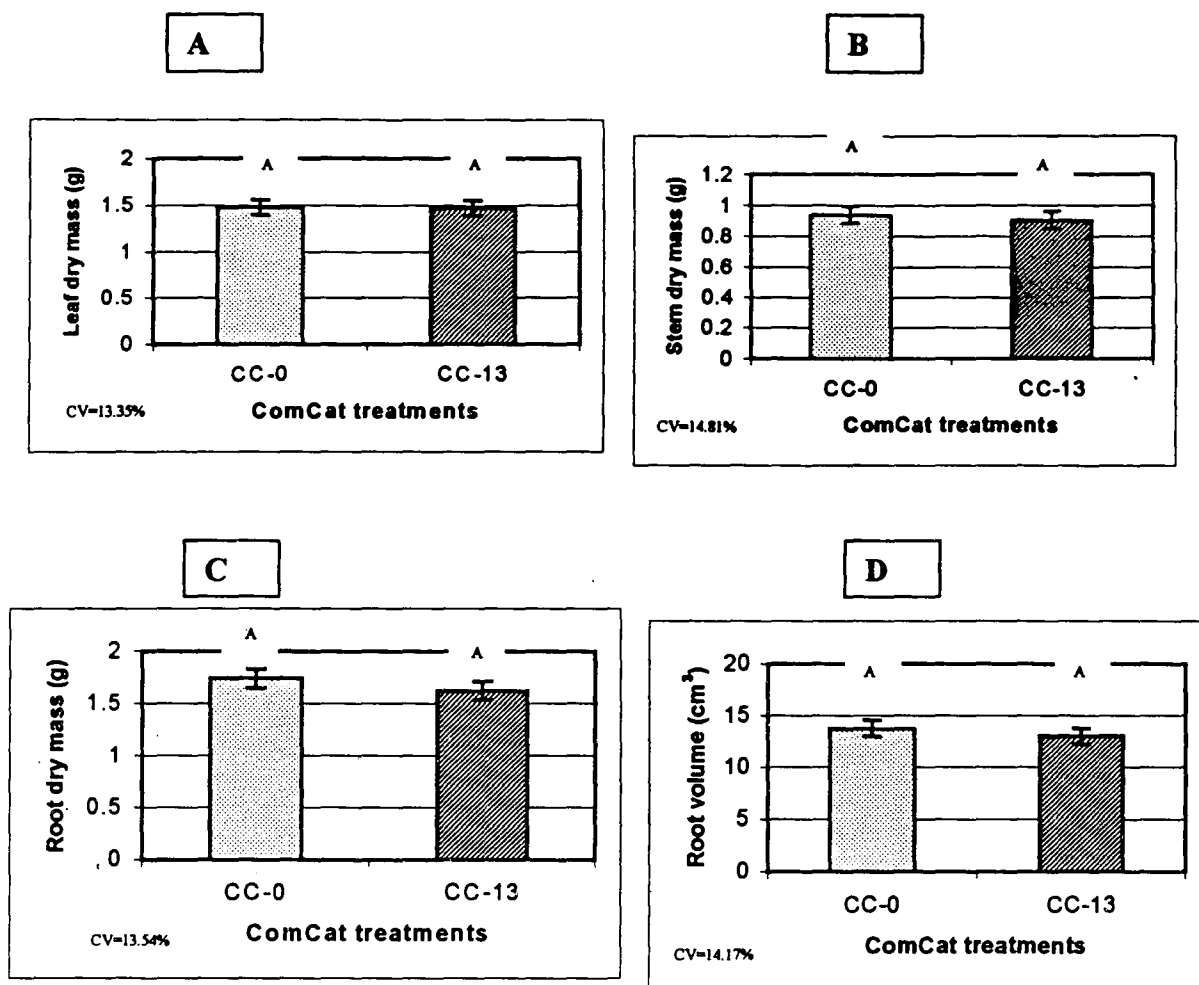
**Figure 4.3:** Influence of cultivar differences on vegetative growth of wheat cultivars as measured at growth stage 19 in terms of mass gain measuring parameters, namely A) leaf dry mass, B) stem dry mass, C) root dry mass and D) root volume. (Similar letters on top of the columns indicate non-significant difference between the mean values at the 5% probability level).

### 4.3.2.3.3 Influence of ComCat treatments

Treatment with ComCat at growth stage 13 (values pooled for both cultivars) had no significant effect on the vegetative growth of either of the two cultivars as measured at growth stage 19, both in terms of size (Figure 4.4A, B and C) and mass (Figures 4.5A, B, C and D) measuring parameters.



**Figure 4.4:** Influence of ComCat treatments on vegetative growth of wheat cultivars as measured at growth stage 19: A) plant height, B) stem thickness and C) leaf area. (Similar letters indicate non-significant difference between mean values at the 5% probability level).



**Figure 4.5:** Influence of ComCat treatments on vegetative growth of wheat cultivars as measured at growth stage 19 in terms of mass gain measuring parameters, namely A) leaf dry mass, B) stem dry mass, C) root dry mass and D) root volume. (Similar letters on top of the columns indicate non-significant difference at the 5% probability level).

### **4.3.3 Vegetative growth characteristics of two Ethiopian wheat cultivars at growth stage 34**

Growth stage 34 represents the middle of the stem elongation period and is characterized by the appearance of a detectable 4<sup>th</sup> node. Like the preceding two growth stages, the variables monitored at this stage involved the two cultivars and ComCat treatment, but at four levels of the latter (i.e. no ComCat application (CC-0), ComCat applied at growth stage 13 (CC-13), 19 (CC-19) and as a double application at growth stages 13 and 19 (CC-13 & 19). The same parameters for measuring size and mass gains, as for the preceding growth stages, were used.

#### **4.3.3.1 Vegetative growth variation in two Ethiopian wheat cultivars, as influenced by treatment with ComCat at growth stages 13 and 19, and measured at growth stage 34.**

The investigation of the effects of each of the eight treatments (raw data of three replications for each treatment) on the vegetative growth of the plants revealed that in the cultivar HAR-2508, application of ComCat appeared to have no effect on vegetative growth in terms of size (plant height, stem thickness and leaf area; Table 4.5). In using mass parameters (Table 4.6), however, ComCat treatment tended to improve growth compared to the untreated controls in HAR-2508. Plants of the same cultivar that received ComCat at growth stage 19 (CC-19) had higher leaf dry mass (+8%) while those that received the CC-13 treatment measured higher in stem dry mass (+17%) those that of the untreated controls (Table 4.6). Similarly, HAR-2508 plants that received the double application of ComCat (CC-13 & 19) measured higher in terms of root volume (+23%) and root dry mass (+59%) those that of the untreated controls (Table 4.6).

**Table 4.5:** Vegetative growth variation, in terms of size gain measuring parameters, in two Ethiopian wheat cultivars as influenced by double treatment with ComCat at growth stages 13 and 19, and measured at growth stage 34.

ComCat treatments	Plant height (cm)		Stem thickness (mm)		Leaf area (cm <sup>2</sup> )	
	HAR-2508	ET-13	HAR-2508	ET-13	HAR-2508	ET-13
CC-0	53.07 ± 3.07 A	60.47 ± 4.32 A	3.58 ± 0.13 A	3.86 ± 0.11 A	591.83 ± 66.23 A	786.23 ± 119.60A
CC-13	49.57 ± 8.36 A	65.30 ± 9.25 A	3.42 ± 0.42 A	3.70 ± 0.17 A	519.73 ± 158.31 A	845.47 ± 118.48A
CC-19	48.93 ± 3.79 A	62.10 ± 4.50 A	3.56 ± 0.17 A	3.62 ± 0.17 A	645.89 ± 117.89 A	873.00 ± 76.58 A
CC-13 & 19	52.93 ± 4.08 A	64.67 ± 9.45 A	3.57 ± 0.17 A	3.69 ± 0.07 A	587.46 ± 139.11 A	796.89 ± 86.22 A
CV (%)	6.57		5.28		16.00	
SE	2.1645		0.1106		65.2201	

Similar letters indicate non-significant differences at the 5% probability level. ± = Standard deviation for three replications

More or less similar trends were observed in the cultivar ET-13 (Tables 4.5 and 4.6), CC-13 and CC-19 treated plants measured higher in terms of plant height (+8%) and leaf area (+11%) than the untreated controls, respectively (Table 4.5). Similarly, in mass gain measuring parameters, CC-19 treated plants showed higher leaf dry mass (+12%) and higher root volume (+34%), while CC-13 treated plants had higher stem dry mass (+24%) compared to their respective untreated controls (Table 4.6). Nevertheless, despite these differences, the analysis of variance did not reveal a statistically significant interaction effect between the cultivars and the ComCat treatments in terms of the above-described parameters.

However, contrary to these observations, a significant interaction effect between the main factors was observed for root dry mass ( $P < 0.05$ ; Table 4.6). Plants of the cultivar

ET-13 that received the double application of ComCat (CC-13 & 19) had a root dry mass that was 97%, 38% and 41% higher than that of the untreated control plants, CC-13 and CC-19 treated plants of the same cultivar, respectively. The root dry mass of CC-13 & 19 treated plants of the cultivar ET-13 was also significantly higher than that of plants of the cultivar HAR-2508 that received ComCat at each of the different growth stages (Table 4.6).

**Table 4.6:** Vegetative growth variation in terms of mass gain measuring parameters in two Ethiopian wheat cultivars, as influenced by treatment with ComCat at growth stages 13 and 19, and measured at growth stage 34.

ComCat treatments	Leaf dry mass (g)		Stem dry mass (g)		Root volume (cm <sup>3</sup> )		Root dry mass (g)	
	HAR-2508	ET-13	HAR-2508	ET-13	HAR-2508	ET-13	HAR-2508	ET-13
CC-0	2.29 ± 0.39 <sub>A</sub>	3.02 ± 0.37 <sub>A</sub>	2.76 ± 0.45 <sub>A</sub>	4.37 ± 1.10 <sub>A</sub>	15.37 ± 0.50 <sub>A</sub>	20.23 ± 2.03 <sub>A</sub>	3.04 ± 0.41 <sub>C</sub>	4.34 ± 1.01 <sub>BC</sub>
CC-13	2.10 ± 0.56 <sub>A</sub>	3.28 ± 0.52 <sub>A</sub>	3.22 ± 0.47 <sub>A</sub>	5.43 ± 0.78 <sub>A</sub>	13.50 ± 2.18 <sub>A</sub>	25.30 ± 4.10 <sub>A</sub>	2.94 ± 0.86 <sub>C</sub>	6.19 ± 0.40 <sub>B</sub>
CC-19	2.47 ± 0.30 <sub>A</sub>	3.37 ± 0.58 <sub>A</sub>	3.09 ± 0.51 <sub>A</sub>	4.84 ± 1.07 <sub>A</sub>	17.43 ± 0.90 <sub>A</sub>	27.07 ± 4.67 <sub>A</sub>	4.24 ± 0.36 <sub>BC</sub>	6.04 ± 0.28 <sub>B</sub>
CC-13 & 19	2.42 ± 0.56 <sub>A</sub>	3.11 ± 0.09 <sub>A</sub>	3.17 ± 0.70 <sub>A</sub>	5.31 ± 0.45 <sub>A</sub>	18.93 ± 1.82 <sub>A</sub>	26.50 ± 2.04 <sub>A</sub>	4.84 ± 1.15 <sub>BC</sub>	8.54 ± 0.78 <sub>A</sub>
CV (%)	17.09		18.84		13.62		15.11	
SE	0.2721		0.4379		1.6517		0.4377	

Different letters indicate significant differences between mean values at the 5% probability level while similar letters indicate non-significant difference; ± = standard deviation for three replications.

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#### 4.3.3.2 Statistical analysis of the correlation between different growth parameters as measured at growth stage 34

In order to investigate the degree of mutual association that might have existed among the growth parameters, a statistical correlation was performed (Table 4.7). The analysis revealed a strong positive association ( $P < 0.01$ ) of plant height with leaf area, leaf dry mass, stem dry mass, root dry mass and root volume. Leaf area was also strongly and positively associated ( $P < 0.01$ ) with leaf dry mass, stem dry mass, root volume and root dry mass.

**Table 4.7:** Coefficients of correlation of different growth parameters measured at growth stage 34.

	Height	Stem thickness	Leaf area	Leaf dry mass	Stem dry mass	Root volume
Stem thickness	0.44*					
Leaf area	0.75**	0.75**				
Leaf dry mass	0.56**	0.74**	0.93**			
Stem dry mass	0.56**	0.42*	0.75**	0.83**		
Root volume	0.68**	0.43*	0.76**	0.73**	0.73**	
Root dry mass	0.56**	0.34 <sup>ns</sup>	0.61**	0.61**	0.64**	0.85**

\*, \*\* = significant correlation at the 5 and 1% probability levels respectively.

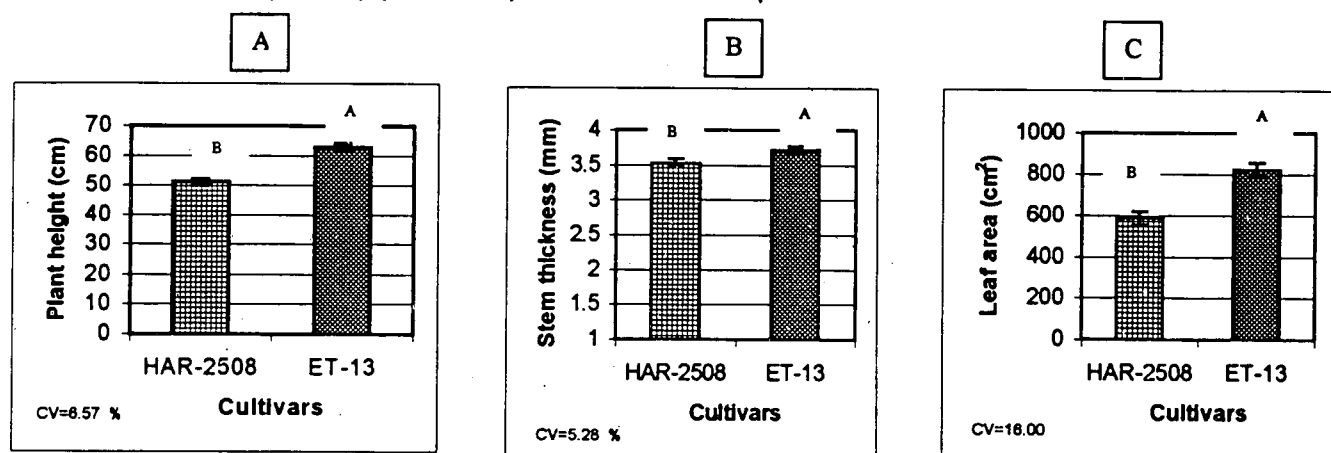
As shown in Table 4.7, leaf growth, measured as leaf dry mass, appeared to be mutually dependent on all of the other parameters. Similarly, root development, measured as root dry mass, was also mutually dependent on all of the other parameters except stem thickness. In the preceding growth stage, root dry mass showed a mutually dependent relationship with only stem dry mass, but the observed differences at this growth stage indicate that the mutual relationship between the roots

and the above ground parts had increased proportionally. Compared to the preceding growth stage (growth stage 19), stronger correlation were observed among more of the growth parameters at growth stage 34, indicating a proportional increase in the correlation between different growth measuring parameters.

#### 4.3.3.3 Statistical analysis of the individual effects of cultivar differences and ComCat treatments on the vegetative growth of two Ethiopian wheat cultivars, as measured at growth stage 34

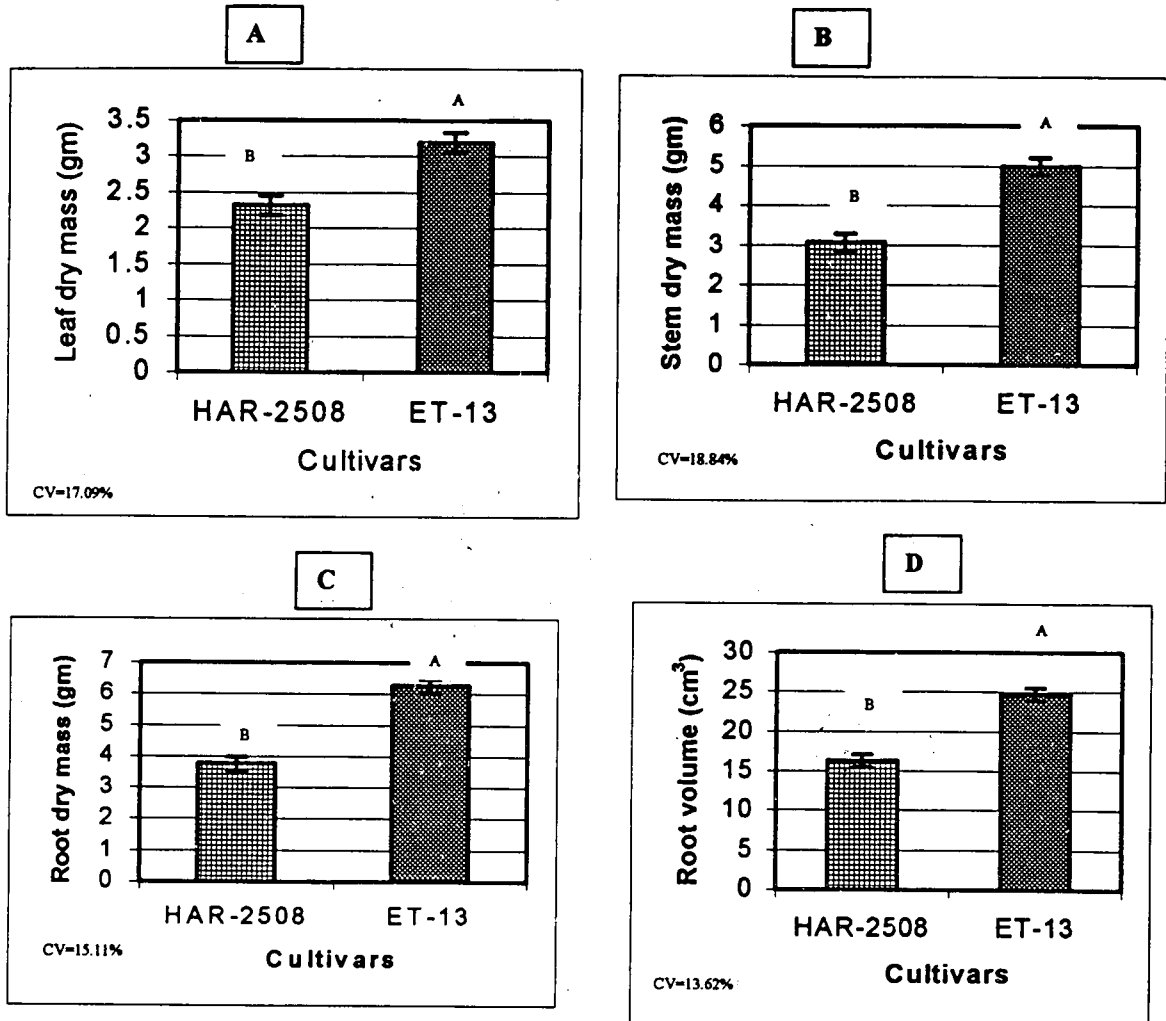
##### 4.3.3.3.1 Influence of cultivar differences (data for ComCat treatments pooled)

With regard to size parameters (Figure 4.6A, B, and C), plants of the cultivar ET-13 were significantly taller (+23%) ( $P < 0.001$ ), had thicker stems (+5%) ( $P < 0.05$ ) and larger leaf area (+41%) ( $P < 0.001$ ) than HAR-2508 plants.



**Figure 4.6:** Vegetative growth of wheat at growth stage 34 as influenced by individual cultivar characteristics in terms of size gain measuring parameters, namely A) plant height, B) stem thickness and C) leaf area. (Different letters on top of the columns indicate significant difference between mean values at the 0.1 % for plant height and leaf area, and at 5% probability level for stem thickness).

Plants of the cultivar ET-13 were significantly superior to HAR-2508 in terms of the mass measuring parameters, namely the dry mass of leaves (+38%; Figure 4.7A), stems (+63%; Figure 4.7B) and roots (+67%; Figure 4.7C) as well as in root volume (+52%; Figure 4.7D) at the 0.1% probability level.

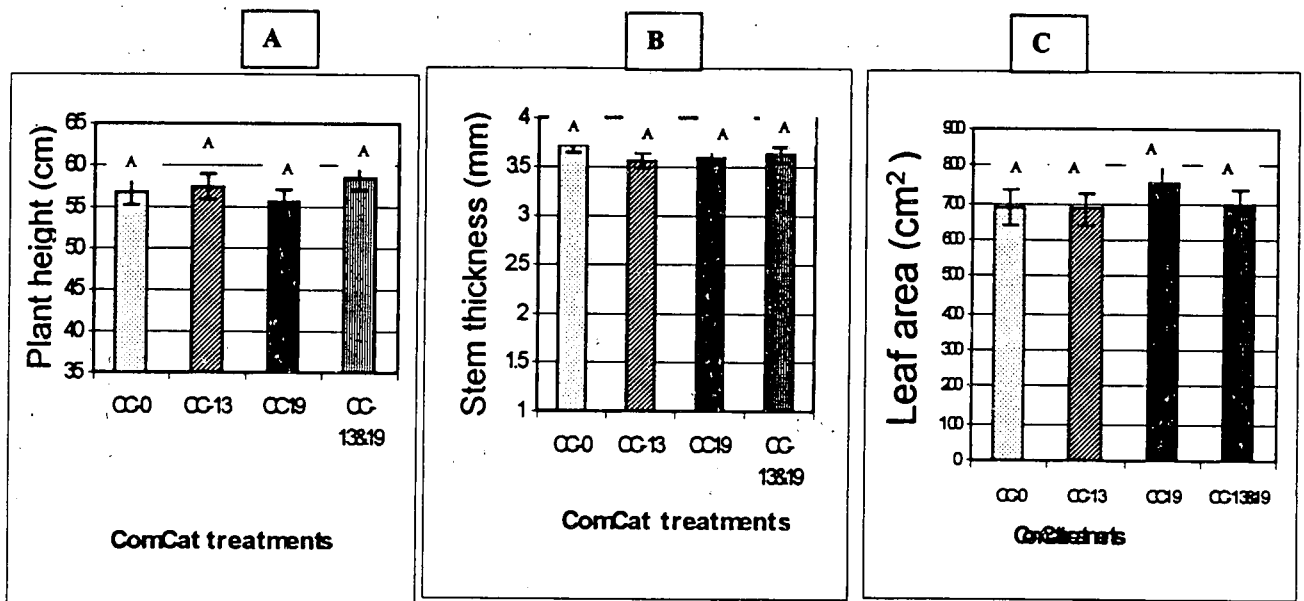


**Figure 4.7:** Vegetative growth of wheat at growth stage 34 as influenced by individual cultivar characteristics in terms of mass gain measuring parameters namely A) leaf dry mass, B) stem dry mass, C) root dry mass and D) root volume. (Different letters on top of the columns indicate significant difference between mean values at the 0.1 % probability level for each of the parameters).

All of the above observations (Figures 4.6 and 4.7) indicated that the two cultivars began showing significant differences in vegetative growth between growth stages 19 and 34, with ET-13 showing the highest growth rate of the two cultivars.

#### 4.3.3.3.2 Influence of ComCat treatments (data for cultivars pooled)

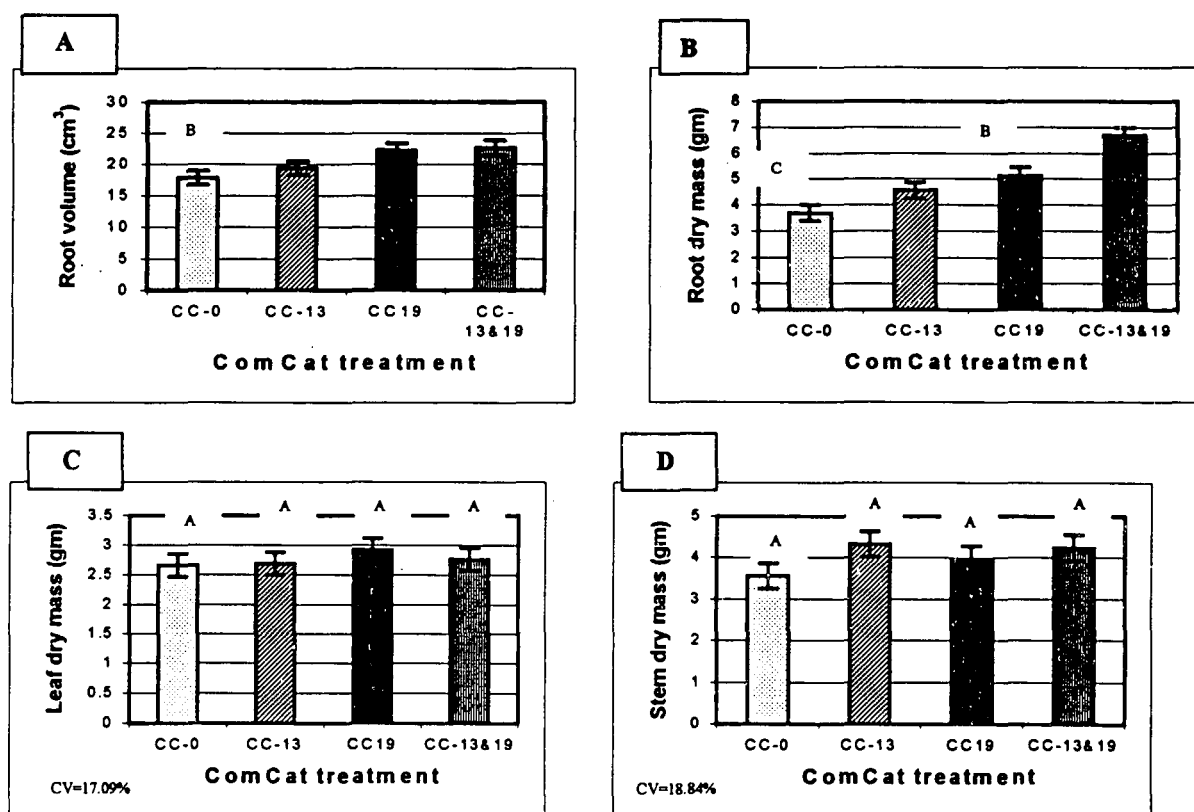
The analysis of variance revealed that the application of ComCat at different growth stages did not contribute significantly to the vegetative growth of wheat in terms of different size gain parameters (Figure 4.8A, B and C) as measured at growth stage 34.



**Figure 4.8:** Vegetative growth of wheat at growth stage 34 as influenced by the individual effects of ComCat treatments in terms of size gain measuring parameters namely A) plant height, B) stem thickness and C) leaf area. (Similar letters on top of the columns indicate non-significant differences between mean values at the 5 % probability level for each of the parameters).

ComCat treatment appeared to enhance root growth (Figures 4.9A and B). Particularly the double application of ComCat significantly increased (+28%) root

volume ( $P < 0.05$ ) (Figure 4.9 A) compared to the untreated controls. However, this was not significantly different from the other ComCat applications (i.e. CC-13 and CC-19). The double ComCat application also significantly ( $P < 0.05$ ) increased root dry mass by 82%, 47% and 30% compared to the untreated controls, CC-13 and CC-19 treated plants, respectively (Figure 4.9B). On the other hand, none of the ComCat treatments showed significantly different effects on leaf dry mass (Figure 4.9C) or stem dry mass (Figure 4.9D) compared to the respective untreated control plants.



**Figure 4.9:** Vegetative growth of wheat at growth stage 34 as influenced by individual effects of ComCat treatment in terms of mass gain measuring parameters namely A) root volume, B) root dry mass, C) leaf dry mass and D) stem dry mass. (Different letters on top of the columns indicate significant difference while similar letters indicate non-significant differences between mean values at the 5 % probability level).

The results presented above (Figures 4.8 and 4.9) clearly showed that the ComCat effect was more related to root development than to that of the other vegetative organs.

#### **4.3.4 Vegetative and early reproductive growth characteristics of two Ethiopian wheat cultivars at growth stages 73-77**

Growth stages 73-77 represent the early grain filling stage also referred to as the milk stage. Unlike the preceding growth stages, three main variables were monitored at this stage, i.e. cultivar differences as well as water and ComCat treatments, at 2, 2, and 4 levels respectively.

When both cultivars reached growth stage 60 (beginning of anthesis), they were subjected to the same magnitude of water stress for two weeks. At the end of the treatment, the plants had reached a developmental stage between growth stages 73 and 77, with HAR-2508 being closer to the last stage in the range.

##### **4.3.4.1 Vegetative growth and early reproductive development of two Ethiopian wheat cultivars as influenced by post anthesis water stress, as well as ComCat treatments, and as measured at growth stages 73-77**

Treatment with ComCat at different growth stages showed no appreciable effects on plant height and stem thickness in both cultivars under both conditions of water treatment (Table 4.8). Similarly, the effect of the water stress treatment *per se*, in terms of these two growth parameters, was non significant.

**Table 4.8:** Influences of post anthesis water stress and ComCat treatments on plant height and stem thickness of two Ethiopian wheat cultivars, as measured at growth stages 73-77.

Treatments (Cultivar X ComCat)	Plant height (cm)		Stem thickness (mm)	
	NWS	WS	NWS	WS
HAR-2508 X CC-0	69.90 ± 3.90 A	69.77 ± 3.71 A	3.21 ± 0.16 A	3.25 ± 0.18 A
HAR-2508 X CC-13	69.97 ± 3.84 A	69.73 ± 5.45 A	3.39 ± 0.17 A	3.19 ± 0.20 A
HAR-2508 X CC-19	71.30 ± 3.62 A	67.80 ± 3.25 A	3.40 ± 0.15 A	3.27 ± 0.17 A
HAR-2508 X CC-13 & 19	69.17 ± 1.33 A	70.60 ± 0.35 A	3.43 ± 0.33 A	3.26 ± 0.03 A
ET-13 X CC-0	94.63 ± 9.93 A	99.83 ± 1.27 A	3.29 ± 0.34 A	2.99 ± 0.25 A
ET-13 X CC-13	100.00 ± 7.82 A	97.73 ± 6.69 A	3.33 ± 0.08 A	3.06 ± 0.09 A
ET-13 X CC-19	99.23 ± 3.65 A	91.17 ± 4.10 A	3.33 ± 0.24 A	3.00 ± 0.05 A
ET-13 X CC-13 & 19	101.47 ± 4.45 A	99.10 ± 5.39 A	3.24 ± 0.05 A	3.06 ± 0.02 A
CV(%)	6.14		5.13	
SE	2.9711		0.0956	

NWS and WS = non-water stress and water stress treatments respectively. Similar letters indicate non-significant differences at the 5% probability level; ± = standard deviation for three replications.

ComCat treatment also did not show any considerable effect on the flag leaf area of plants of the cultivar HAR-2508 under both conditions of water treatment (Table 4.9). However, this was not the case of the cultivar ET-13, where plants that received the double ComCat application (CC-13 & 19) under NWS conditions and those that received ComCat at growth stage 19 (CC-19) under WS conditions, showed increased flag leaf areas (i.e. +21% and +14%, respectively) compared to their respective untreated control plants.

**Table 4.9:** Influences of post anthesis water stress and ComCat treatments on leaf areas of two Ethiopian wheat cultivars, as measured at growth stages 73-77.

Treatments (Cultivar X ComCat)	Flag leaf area (cm <sup>2</sup> )		Total leaf area (cm <sup>2</sup> )	
	NWS	WS	NWS	WS
HAR-2508 X CC-0	54.12 ± 4.41 A	57.52 ± 2.57 A	340.41 ± 35.52 A	231.39 ± 17.24 A
HAR-2508 X CC-13	48.81 ± 7.37 A	49.64 ± 7.49 A	352.01 ± 31.41 A	242.68 ± 39.69 A
HAR-2508 X CC-19	55.25 ± 9.10 A	53.74 ± 3.01 A	310.83 ± 20.51 A	269.62 ± 46.32 A
HAR-2508 X CC-13 & 19	56.05 ± 3.69 A	60.13 ± 4.04 A	354.75 ± 13.87 A	232.59 ± 13.64 A
ET-13 X CC-0	39.41 ± 5.43 A	43.45 ± 3.13 A	435.16 ± 61.22 A	260.00 ± 26.00 A
ET-13 X CC-13	46.09 ± 6.24 A	43.37 ± 5.91 A	472.53 ± 53.67 A	271.25 ± 43.84 A
ET-13 X CC-19	45.68 ± 5.19 A	49.34 ± 4.54 A	441.92 ± 43.48 A	314.49 ± 43.48 A
ET-13 X CC-13 & 19	47.50 ± 7.43 A	39.86 ± 7.72 A	533.16 ± 97.62 A	279.09 ± 59.75 A
CV(%)	11.83		13.22	
SE	3.3721		25.489	

Similar letters indicate non-significant difference at the 5% probability level. ± = Standard deviation for three replications.

No appreciable effect of the ComCat treatment was observed on the total leaf area of the cultivar HAR-2508 under NWS conditions (Table 4.9). However, the water stress treatment reduced the total leaf area of untreated plants of this cultivar by 32% while the CC-19 ComCat treatment tended to prevent this reduction to a large extent.

In ET-13 plants, the double application of ComCat (CC-13 & 19) increased the total leaf area by 18% under NWS conditions (Table 4.9). Similarly, the water stress treatment reduced the total leaf area in untreated plants by > 40% in this cultivar while the CC-19 ComCat treatment again tended to prevent this reduction albeit not as marked as in the case of HAR-2508. However, none of the above differences between controls and ComCat treated plants were statistically significant for both levels of water treatment according to the analysis of variance procedure.

The dry masses of leaves of both cultivars were not appreciably affected by the water stress treatment and the different ComCat applications had no significant accentuating or alleviating effect (Table 4.10). However, the stem dry mass of the cultivar HAR-2508 was reduced by all ComCat applications at different growth stages under both conditions of water treatment (Table 4.10).

**Table 4.10:** Influences of post anthesis water stress and ComCat treatments on dry masses of leaves and stems of two Ethiopian wheat cultivars, as measured at growth stages 73-77.

Treatments (Cultivar X ComCat)	Leaf dry mass (g)		Stem dry mass (g)	
	NWS	WS	NWS	WS
HAR-2508 X CC-0	2.55 ± 0.27 A	2.55 ± 0.28 A	7.71 ± 0.44 A	6.88 ± 1.30 A
HAR-2508 X CC-13	2.29 ± 0.18 A	2.38 ± 0.58 A	6.13 ± 0.54 A	6.40 ± 2.63 A
HAR-2508 X CC-19	2.56 ± 0.23 A	2.54 ± 0.15 A	7.52 ± 0.30 A	6.24 ± 0.68 A
HAR-2508 X CC-13 & 19	2.66 ± 0.31 A	2.55 ± 0.21 A	7.31 ± 1.73 A	6.65 ± 0.98 A
ET-13 X CC-0	3.25 ± 0.16 A	2.99 ± 0.29 A	11.23 ± 2.54 A	9.52 ± 1.39 A
ET-13 X CC-13	3.43 ± 0.16 A	3.02 ± 0.06 A	12.30 ± 1.89 A	9.49 ± 0.47 A
ET-13 X CC-19	3.28 ± 0.24 A	2.86 ± 0.10 A	12.04 ± 2.08 A	8.49 ± 0.38 A
ET-13 X CC-13 & 19	3.37 ± 0.20 A	3.10 ± 0.07 A	13.13 ± 1.06 A	9.66 ± 0.50 A
CV(%)		7.60		11.70
SE		0.1247		0.5941

NWS and WS = non-water stress and water stress treatments respectively. Similar letters indicate non-significant difference at the 5% probability level; ± = standard deviation for three replications.

In contradiction, all ComCat treatments tended to increase the stem dry mass of ET-13 under NWS conditions (Table 4.10). However, although the water stress treatment reduced the stem dry mass in untreated controls by 15% in ET-13, ComCat application did not accentuate or alleviate this reduction significantly.

On the other hand, ComCat application at different growth stages tended to reduce both the root volume and root dry mass in HAR-2508 under NWS conditions (Table

4.11) at growth stages 73-77. However, under WS conditions the double ComCat application (CC-13 & 19) resulted in increases in both root volume (+23%) and root dry mass (+30%) in HAR-2508 compared to the untreated controls. This indicated that the double application of ComCat (CC-13 & 19) tended to minimize the reducing effect on root growth under water stress conditions, as measured at growth stages 73-77, in HAR-2508.

**Table 4.11:** Influences of post anthesis water stress and ComCat treatments on root volume, root dry mass and spike dry mass of two Ethiopian wheat cultivars, as measured at growth stages 73-77.

Treatments (Cultivar X ComCat )	Root volume (cm <sup>3</sup> )		Root dry mass (g)		Spike dry mass (g)	
	NWS	WS	NWS	WS	NWS	WS
HAR-2508 X CC-0	27.67 ±1.94 A	21.73 ±0.71 A	11.69 ±0.94A	9.46±1.33 A	6.82 ± 6.82A	6.17 ± 0.43A
HAR-2508 X CC-13	27.03 ±3.01 A	20.57 ±7.43 A	10.50 ±3.28A	7.45 ±3.71 A	5.08 ±0.83A	5.65 ±2.17A
HAR-2508 X CC-19	25.10 ±1.10 A	22.07 ±2.45 A	8.01 ±1.06 A	7.97 ±0.67 A	7.00 ±0.78A	5.78 ±0.68A
HAR-2508 X CC-13 & 19	26.60 ±3.24A	26.77 ±4.22 A	10.39 ±0.39A	12.31 ±1.11 A	5.89 ±1.22A	6.92 ±0.85A
ET-13 X CC-0	26.53 ±2.02A	22.73 ±1.89 A	12.75 ±3.36A	8.97 ±0.51 A	8.05 ±0.72A	8.08 ±0.56A
ET-13 X CC-13	31.53 ±3.59 A	25.20 ±4.78 A	15.42 ±4.32A	10.28 ±3.34 A	7.80 ±0.81A	8.04 ±0.68A
ET-13 X CC-19	26.80 ±3.10 A	24.70 ±4.62 A	11.90 ±1.23 A	10.96 ±4.53 A	8.12 ±1.71A	5.90 ±0.48A
ET-13 X CC-13& 19	29.33 ±1.50 A	26.43 ±1.03A	12.09 ±1.98 A	12.60 ±1.36 A	7.86 ±0.76A	8.40 ±0.57A
CV(%)	11.21		23.28		12.73	
SE	1.6616		1.4519		0.5123	

Similar letters indicate non-significant difference at the 5% probability level. ± = Standard deviation for three replications.

In contradiction, some ComCat treatments tended to improve the volume and dry mass of roots of the cultivar ET-13 under both levels of water treatment (Table 4.11). The earliest application of ComCat (CC-13) increased the root volume by 19% and the

root dry mass by 21% in ET-13 plants under NWS conditions compared to the untreated control plants, while the double application (CC-13 & 19) increased the root volume by 16% and the root dry mass by 40% in the same cultivar under WS conditions (Table 4.11). This indicated that ComCat tended to minimize the reducing effect water deficit stress had on root growth in ET-13.

Spike dry mass tended to be reduced by ComCat application, particularly by the early treatment (CC-13) in the cultivar HAR-2508 under NWS, while a slight increase was observed under the influence of the CC-19 treatment. However, under WS conditions the double application of ComCat (CC-13 & 19) resulted in a 12% increase in spike dry mass in the same cultivar. On the other hand, ComCat application at different growth stages in the cultivar ET-13 had no alleviating or accentuating effect on the spike dry mass under both conditions of water treatment (Table 4.11).

Despite some of the substantial differences observed in the vegetative growth of the two cultivars due to the water stress and the ComCat treatments at different growth stages, the analysis of variance revealed that all the differences were non-significant at the 5% probability level.

#### **4.3.4.2 Statistical analysis of the correlation between different growth parameters as measured at growth stages 73-77**

This analysis revealed similar trends as was the case among many of the parameters correlated at the preceding growth stage, but with some variations. As shown in Table 4.12, plant height strongly correlated with all parameters ( $P < 0.01$ ) except with stem thickness. At the preceding growth stage, there was a strong correlation between these two parameters. Stem thickness, on the other hand, significantly and strongly correlated with flag leaf area ( $P < 0.05$ ) and root volume ( $P < 0.01$ ). Flag leaf area also showed significant but negative correlation with plant height, leaf dry mass, stem dry mass and spike dry mass. It, however, strongly and positively correlated ( $p < 0.05$ ) with stem thickness. No significant correlation between flag leaf area and total leaf area,

root volume and root dry mass was observed. The growth of the reproductive organ, i.e. the spike, appeared to be closely related to all the other parameters, except stem thickness and total leaf area. The correlation analysis also indicated that the growth of roots was closely dependent on the growth of the above ground parts. This was shown by the very strong positive association between the root growth parameters, i.e. root volume and root dry mass, and most of the other above ground parts, with the exception of stem thickness and flag leaf area.

**Table 4.12 :** Coefficients of correlation of different growth parameters  
measured at growth stage 73-77.

	Height	Stem thickness	Flag leaf area	Total leaf area	Leaf dry mass	Stem dry mass	Root volume	Root dry mass
Stem thickness	-0.23 <sup>ns</sup>							
Flag leaf area	-0.61 <sup>**</sup>	0.31 <sup>*</sup>						
Total leaf area	0.43 <sup>**</sup>	0.21 <sup>ns</sup>	-0.20 <sup>ns</sup>					
Leaf dry mass	0.79 <sup>**</sup>	0.11 <sup>ns</sup>	-0.41 <sup>**</sup>	0.57 <sup>**</sup>				
Stem dry mass	0.82 <sup>**</sup>	0.19 <sup>ns</sup>	-0.41 <sup>**</sup>	0.61 <sup>**</sup>	0.91 <sup>**</sup>			
Root volume	0.30 <sup>*</sup>	0.37 <sup>**</sup>	0.03 <sup>ns</sup>	0.54 <sup>**</sup>	0.53 <sup>**</sup>	0.55 <sup>**</sup>		
Root dry mass	0.40 <sup>**</sup>	0.12 <sup>ns</sup>	-0.02 <sup>ns</sup>	0.48 <sup>**</sup>	0.49 <sup>**</sup>	0.48 <sup>**</sup>	0.82 <sup>**</sup>	
Spike dry mass	0.69 <sup>**</sup>	0.09 <sup>ns</sup>	-0.33 <sup>*</sup>	0.20 <sup>ns</sup>	0.76 <sup>**</sup>	0.79 <sup>**</sup>	0.41 <sup>**</sup>	0.36 <sup>**</sup>

\*, \*\* = significant correlation at 5, and 1% probability levels respectively. ns = non-significant correlation at the 5% probability level.

**4.3.4.3 Statistical analysis of the interaction effects of any two of the three main factors (cultivar differences as well as water and ComCat treatments) on vegetative and early reproductive development of two Ethiopian wheat cultivars, as measured at growth stages 73-77**

**4.3.4.3.1 Statistical analysis of water X cultivar interaction**

**Table 4.13:** Influence of water X cultivar interaction on vegetative and early reproductive development of two Ethiopian wheat cultivars measured by using different parameters, as measured at growth stages 73-77.

Growth parameters	HAR-2508		ET-13		CV (%)	SE
	NWS	WS	NWS	WS		
Plant height (cm)	70.08 <sup>A</sup>	69.48 <sup>A</sup>	98.83 <sup>A</sup>	96.96 <sup>A</sup>	6.14	1.4856
Stem thickness (mm)	3.36 <sup>A</sup>	3.24 <sup>A</sup>	3.30 <sup>A</sup>	3.03 <sup>A</sup>	5.13	0.0478
Flag leaf area (cm <sup>2</sup> )	53.56 <sup>A</sup>	55.26 <sup>A</sup>	44.67 <sup>A</sup>	44.01 <sup>A</sup>	11.83	1.6861
Total leaf area (cm <sup>2</sup> )	339.50 <sup>B</sup>	244.07 <sup>C</sup>	470.69 <sup>A</sup>	281.21 <sup>C</sup>	13.22	12.7445
Leaf dry mass (g)	2.51 <sup>C</sup>	2.50 <sup>C</sup>	3.33 <sup>A</sup>	3.02 <sup>B</sup>	7.6	0.0624
Stem dry mass (g)	7.17 <sup>C</sup>	6.54 <sup>C</sup>	12.18 <sup>A</sup>	9.29 <sup>B</sup>	11.70	0.2971
Root volume (cm <sup>3</sup> )	26.60 <sup>A</sup>	22.78 <sup>A</sup>	28.55 <sup>A</sup>	24.77 <sup>A</sup>	11.21	0.8303
Root dry mass (g)	10.17 <sup>A</sup>	9.30 <sup>A</sup>	13.04 <sup>A</sup>	10.70 <sup>A</sup>	23.28	0.7259
Spike dry mass (g)	6.20 <sup>A</sup>	6.13 <sup>A</sup>	7.95 <sup>A</sup>	7.60 <sup>A</sup>	12.73	0.2562

Different letters indicate significant difference while similar letters indicate non-significant difference at the 5% probability level.

Statistical analysis of this interaction effect (data pooled for all ComCat treatments) revealed that the cultivar ET-13 performed better in terms of vegetative growth compared to the cultivar HAR-2508 under NWS conditions. The former cultivar was

superior in plant height (+41%), total leaf area (+39%), leaf dry mass (+33%), stem dry mass (+70%), root volume (+7%), root dry mass (+28%) and spike dry mass (+28%) (Table 4.13) to HAR-2508. On the other hand, under the same water treatment, HAR-2508 was slightly superior in stem thickness (+2%) but considerably superior in flag leaf area (+20%) to ET-13.

Trends were similar under water stress conditions, where the cultivar ET-13 again excelled in plant height (+40%), total leaf area (+15%), leaf dry mass (+21%), stem dry mass (+42%), root volume (+9%), root dry mass (+15%) and spike dry mass (+24%). Under the same water treatment, the cultivar HAR-2508 was again superior in stem thickness (+7%) and flag leaf area (26%).

According to the analysis of variance, the interaction between the two factors was statistically significant ( $P < 0.05$ ) only for total leaf area, leaf dry mass and stem dry mass (Table 4.13). Furthermore, in the parameters where the two factors showed significant interaction, the highest interaction effects were consistently observed between the cultivar ET-13 and the non-water stress treatment, while the least interaction effects were observed between the cultivar HAR-2508 and the water stress treatment (Table 4.13). The water deficit stress appeared to have caused more reductions in ET-13 in terms of total leaf area and leaf dry mass than in HAR-2508. The reduction in total leaf area, due to the water stress condition, was 28% and 40% in HAR-2508 and ET-13 respectively. The reduction in terms of leaf dry mass was 9% in ET-13 while no reduction occurred in HAR-2508.

#### 4.3.4.3.2 Statistical analysis of water X ComCat treatment interaction

**Table 4.14:** Influence of water X ComCat treatment interaction on vegetative and early reproductive development of two Ethiopian wheat cultivars measured by using different parameters, and as measured at growth stages 73-77.

Interaction (water x ComCat)	Plant height (cm)	Stem thick- ness (mm)	Flag leaf area (cm <sup>2</sup> )	Total leaf area (cm <sup>2</sup> )	Leaf dry mass (g)	Stem dry mass (g)	Root volume (cm <sup>3</sup> )	Root dry mass (g)	Spike dry mass (g)
Non WS X CC-0	82.27 <sub>A</sub>	3.25 <sub>A</sub>	46.76 <sub>A</sub>	387.79 <sub>B</sub>	2.90 <sub>A</sub>	9.47 <sub>A</sub>	27.10 <sub>A</sub>	12.22 <sub>AB</sub>	7.43 <sub>A</sub>
Non WS X CC-13	84.98 <sub>A</sub>	3.36 <sub>A</sub>	47.45 <sub>A</sub>	412.27 <sub>AB</sub>	2.86 <sub>A</sub>	9.23 <sub>A</sub>	29.28 <sub>A</sub>	12.96 <sub>A</sub>	6.44 <sub>A</sub>
Non WS X CC-19	85.27 <sub>A</sub>	3.37 <sub>A</sub>	50.47 <sub>A</sub>	376.37 <sub>B</sub>	2.92 <sub>A</sub>	9.78 <sub>A</sub>	25.95 <sub>A</sub>	10.00 <sub>BC</sub>	7.56 <sub>A</sub>
Non WS X CC-13 & 19	85.32 <sub>A</sub>	3.34 <sub>A</sub>	51.78 <sub>A</sub>	443.96 <sub>A</sub>	3.01 <sub>A</sub>	10.22 <sub>A</sub>	27.97 <sub>A</sub>	11.24 <sub>ABC</sub>	6.88 <sub>A</sub>
WS X CC-0	84.80 <sub>A</sub>	3.12 <sub>A</sub>	50.49 <sub>A</sub>	245.70 <sub>D</sub>	2.77 <sub>A</sub>	8.20 <sub>A</sub>	22.23 <sub>A</sub>	9.21 <sub>C</sub>	7.12 <sub>A</sub>
WS X CC-13	83.73 <sub>A</sub>	3.13 <sub>A</sub>	46.50 <sub>A</sub>	256.96 <sub>CD</sub>	2.76 <sub>A</sub>	7.95 <sub>A</sub>	22.88 <sub>A</sub>	8.87 <sub>C</sub>	6.84 <sub>A</sub>
WS X CC-19	79.48 <sub>A</sub>	3.14 <sub>A</sub>	51.54 <sub>A</sub>	292.05 <sub>C</sub>	2.70 <sub>A</sub>	7.37 <sub>A</sub>	23.38 <sub>A</sub>	9.46 <sub>C</sub>	5.84 <sub>A</sub>
WS X CC-13 & 19	84.85 <sub>A</sub>	3.16 <sub>A</sub>	50.00 <sub>A</sub>	255.84 <sub>CD</sub>	2.82 <sub>A</sub>	8.16 <sub>A</sub>	26.6 <sub>A</sub>	12.45 <sub>AB</sub>	7.66 <sub>A</sub>
C.V (%)	6.14	5.13	11.83	13.22	7.6	11.7	11.21	23.28	12.73
SE	2.1009	0.0676	2.3845	18.0235	0.0882	0.4201	1.175	1.0266	0.3623

Different letters at the front of mean values indicate significant difference at the 10 % probability level, while similar letters indicate otherwise.

This interaction (data pooled for cultivars) was statistically significant ( $P < 0.1$ ) only in terms of total leaf area and root dry mass (Table 4.14). Generally, larger leaf areas were observed under the non-water stress condition with those plants that received

the double application of ComCat (CC-13 & 19) having significantly ( $P < 0.1$ ) larger leaf areas than each of all the other plants treated with ComCat under both levels of water treatment. The smallest leaf area was observed in the untreated control plants grown under the water deficit stress condition.

The early ComCat application (CC-13) particularly under the non-water stress conditions tended to enhance root growth. Under the water stress condition, the double application of ComCat (CC-13 & 19) yielded the highest root dry mass that was significantly ( $P < 0.1$ ) higher than that of the other ComCat treated plants under both the non-water stress and the water deficit stress conditions.

The vegetative growth of the plants, in terms of the remaining parameters, appeared to have not been considerably affected by the water X ComCat interaction, except that a general reduction trend in growth due to the water stress was observed in most of the growth parameters (Table 4.14). The analysis of variance also revealed the interaction effect, in terms of all the remaining parameters, to be non-significant at the 5% probability level.

#### 4.3.4.3.3 Statistical analysis of cultivar X ComCat treatment interaction

**Table 4.15:** Influence of cultivar X ComCat treatment interaction on vegetative and early reproductive development of two Ethiopian wheat cultivars, measured by using different parameters, and as measured at growth stages 73-77.

Interaction (cultivars x ComCat)	Plant height (cm)	Stem thickness (mm)	Flag leaf area (cm <sup>2</sup> )	Total leaf area (cm <sup>2</sup> )	Leaf dry mass (g)	Stem dry mass	Root volume (cm <sup>3</sup> )	Root dry mass (g)	Spike dry mass(g)
HAR-2508 X CC-0	69.83 A	3.23 A	55.82 A	285.90 A	2.55 C	7.29 A	24.70 A	10.57 A	6.49 B
HAR-2508 X CC-13	69.85 A	3.29 A	49.23 A	297.34 A	2.33 D	6.26 A	23.80 A	8.96 A	5.37 C
HAR-2508 X CC-19	69.55 A	3.34 A	54.50 A	290.22 A	2.55 C	6.88 A	23.58 A	8.03 A	6.39 B
HAR-2508 X CC-13 & 19	69.88 A	3.35 A	58.09 A	293.67 A	2.60 C	6.98 A	26.68 A	11.35 A	6.41 B
ET-13 X CC-0	97.23 A	3.14 A	41.43 A	347.58 A	3.12 AB	10.38 A	24.63 A	10.86 A	8.06 A
ET-13 X CC-13	98.87 A	3.19 A	44.73 A	371.89 A	3.29 A	10.91 A	28.37 A	12.85 A	7.92 A
ET-13 X CC-19	95.20 A	3.17 A	47.51 A	378.21 A	3.07 B	10.27 A	25.75 A	11.43 A	7.01 B
ET-13 X CC-13 & 19	100.28 A	3.15 A	43.68 A	406.13 A	3.24 AB	11.39 A	27.88 A	12.34 A	8.13 A
C.V (%)	6.14	5.13	11.83	13.22	7.6	11.7	11.21	23.28	12.73
SE	2.1009	0.0676	2.3845	18.0235	0.0882	0.4201	1.175	1.0266	0.3623

Different letters at the front of means indicate significant difference at the 5% probability level, while similar letters indicate other wise.

This interaction (values pooled over both levels of water treatment) was significant ( $P < 0.05$ ) only in terms of leaf dry mass and spike dry mass (Table 4.15). The earliest application of ComCat (CC-13) in ET-13 plants produced the highest leaf dry mass compared to each of the other ComCat applications in both cultivars. However, it was not significantly higher than that of untreated controls, as well as CC-13 & 19 treated ET-13 plants.

ComCat application also tended to enhance the development of spikes. Particularly the double application of ComCat in ET-13 plants produced spikes which were significantly higher in dry mass than those of the cultivar HAR-2508 that received ComCat at different growth stages, as well as those from ET-13 plants that received ComCat at growth stage 19 (Table 4.15). The above observations appeared to indicate lack of consistency in the effects of ComCat treatments in as much as that the same ComCat treatments did not produce the highest dry mass values for both leaves and spikes.

According to the analysis of variance, application of ComCat at the different growth stages in both cultivars did not appear to significantly enhance vegetative growth in terms of the other growth parameters (other than leaf and spike dry mass; Table 4.15). However, in most of the remaining parameters a general tendency to produce relatively higher values in ET-13 plants, compared to that in HAR-2508 plants, was observed.

#### 4.3.4.4 Effects of cultivar differences as well as treatments of water and ComCat on vegetative and early reproductive development of two Ethiopian wheat cultivars, as measured at growth stages 73-77

##### 4.3.4.4.1 Influence water treatment

**Table 4.16:** Statistical analysis of the individual effects of water treatment on vegetative and early reproductive development of two Ethiopian wheat cultivars, as measured at growth stages 73-77.

Water levels	Plant height (cm)	Stem thickness (mm)	Flag leaf area (cm <sup>2</sup> )	Total leaf area (cm <sup>2</sup> )	Leaf dry mass (g)	Stem dry mass (g)	Root volume (cm <sup>3</sup> )	Root dry mass (g)	Spike dry mass (g)
Non-water stress	84.46 <sub>A</sub>	3.33 <sub>A</sub>	49.11 <sub>A</sub>	405.10 <sub>A</sub>	2.92 <sub>A</sub>	9.67 <sub>A</sub>	27.58 <sub>A</sub>	11.61 <sub>A</sub>	7.08 <sub>A</sub>
Water stress	83.22 <sub>A</sub>	3.13 <sub>B</sub>	49.63 <sub>A</sub>	262.64 <sub>B</sub>	2.76 <sub>B</sub>	7.92 <sub>B</sub>	23.78 <sub>B</sub>	10.00 <sub>B</sub>	6.87 <sub>A</sub>
CV (%)	6.14	5.13	11.83	13.22	7.6	11.70	11.21	23.28	12.73
SE	1.0504	0.0338	1.1922	9.0117	0.0441	0.2100	0.5875	0.5133	0.1811

Similar letters in front of mean values indicate lack of significant difference at the 5% probability level, while the different letters indicate significant difference at the 0.1% probability level for all parameters, except for root dry mass, where differences were significant at the 5% probability level.

Significant differences were observed between the two levels of water treatment (data pooled for cultivars and ComCat treatments) in several of the size and mass measuring parameters, except in plant height, flag leaf area and spike dry mass (Table 4.16). The two week post anthesis water stress treatment resulted in

significant reductions in stem thickness (-6%;  $P < 0.001$ ), total leaf area (-35%;  $P < 0.001$ ), leaf dry mass (-5%;  $P < 0.05$ ), Stem dry mass (-18%;  $P < 0.001$ ), root volume (-14%;  $P < 0.001$ ) and root dry mass (-14%;  $P < 0.05$ ) (Table 4.16). Plant height, flag leaf area and spike dry mass, appeared to be less affected by the water stress treatment.

#### 4.3.4.4.2 Influence of cultivar differences

**Table 4.17:** Statistical analysis of the individual effects of cultivar differences on vegetative and early reproductive development of two Ethiopian wheat cultivars, as measured at growth stages 73-77.

Cultivars	Plant height (cm)	Stem thickness (mm)	Flag leaf area (cm <sup>2</sup> )	Total leaf area (cm <sup>2</sup> )	Leaf dry mass (g)	Stem dry mass (g)	Root volume (cm <sup>3</sup> )	Root dry mass (g)	Spike dry mass (g)
HAR-2508	69.78 <sub>B</sub>	3.30 <sub>A</sub>	54.41 <sub>A</sub>	291.78 <sub>B</sub>	2.51 <sub>B</sub>	6.85 <sub>B</sub>	24.69 <sub>B</sub>	9.73 <sub>B</sub>	6.16 <sub>B</sub>
ET-13	97.90 <sub>A</sub>	3.16 <sub>B</sub>	44.34 <sub>B</sub>	375.95 <sub>A</sub>	3.18 <sub>A</sub>	10.74 <sub>A</sub>	26.66 <sub>A</sub>	11.87 <sub>A</sub>	7.78 <sub>A</sub>
CV (%)	6.14	5.13	11.83	13.22	7.6	11.70	11.21	23.28	12.73
SE	1.0504	0.0338	1.1922	9.0117	0.0441	0.2100	0.5875	0.5133	0.1811

Similar letters behind of mean values indicate lack of significant difference at the 5% probability level. The different letters indicate significant differences at the 0.1% probability level for the other parameters, with the exception of root volume, root dry mass and spike dry mass, where differences were significant at the 5%, 1% and 1% probability levels respectively.

Statistical analysis of the cultivar effect revealed significant differences in terms of all size and mass measuring parameters (Table 4.17). Plants of the cultivar HAR-2508 had significantly thicker stems (+4%) and larger flag leaf area (+23%) compared to

those of ET-13. Plants of ET-13, on the other hand, were superior to HAR-2508 in all the other parameters, i.e. plant height (+40%), total leaf area (+29%), leaf dry mass (+27%), stem dry mass (+57%), root volume (+8%), root dry mass (+22%) and spike dry mass (+26%) (Table 4.17).

#### 4.3.4.4.3 Influence of the ComCat treatments

**Table 4.18:** Statistical analysis of the individual effects of the ComCat treatments on vegetative and early reproductive development of two Ethiopian wheat cultivars, as measured at growth stages 73-77.

ComCat levels	Plant height (cm)	Stem thickness (mm)	Flag leaf area (cm <sup>2</sup> )	Total leaf area (cm <sup>2</sup> )	Leaf dry mass (g)	Stem dry mass (g)	Root volume (cm <sup>3</sup> )	Root dry mass (g)	Spike dry mass (g)
CC-0	83.53 <sub>A</sub>	3.18 <sub>A</sub>	48.62 <sub>A</sub>	316.74 <sub>A</sub>	2.84 <sub>A</sub>	8.84 <sub>A</sub>	24.67 <sub>B</sub>	10.72 <sub>A</sub>	7.27 <sub>A</sub>
CC-13	84.36 <sub>A</sub>	3.24 <sub>A</sub>	46.98 <sub>A</sub>	334.62 <sub>A</sub>	2.81 <sub>A</sub>	8.59 <sub>A</sub>	26.08 <sub>AB</sub>	10.91 <sub>A</sub>	6.64 <sub>A</sub>
CC-19	82.38 <sub>A</sub>	3.25 <sub>A</sub>	51.01 <sub>A</sub>	334.21 <sub>A</sub>	2.81 <sub>A</sub>	8.57 <sub>A</sub>	24.67 <sub>B</sub>	9.73 <sub>A</sub>	6.70 <sub>A</sub>
CC-13 & 19	85.08 <sub>A</sub>	3.25 <sub>A</sub>	50.89 <sub>A</sub>	349.90 <sub>A</sub>	2.92 <sub>A</sub>	9.19 <sub>A</sub>	27.28 <sub>A</sub>	11.85 <sub>A</sub>	7.27 <sub>A</sub>
CV (%)	6.14	5.13	11.83	13.22	7.6	11.70	11.21	23.28	12.73
SE	1.4856	0.0478	1.6861	12.7445	0.0624	0.2971	0.8309	0.7259	0.2562

The different letters indicate significant differences at the 10% probability level, while similar letters in front indicate non-significant differences.

Unlike the individual effects of water treatments and cultivar differences, application of ComCat at the different growth stages appeared to have had no significant individual effect on vegetative growth of the plants as measured in terms of most of the growth parameters. The four levels of ComCat treatment did not contribute to any significant differences (Table 4.18), in all but one parameter (i.e. root volume). The double application of ComCat yielded the highest root volume ( $P < 0.10$ ) compared to the other two levels of treatment as well as the untreated control. ComCat applied at growth stage 13 gave the second highest root volume that was slightly higher than that of the control and the application at growth stage 19. However, in all of the other parameters, albeit statistically non-significant, at least one of the ComCat applications consistently resulted in higher values compared to that of the untreated control plants (Table 4.18).

#### **4.3.5 Summary of the growth patterns of two Ethiopian wheat cultivars as measured at different growth stages \***

In order to characterize the vegetative growth patterns exhibited by the two cultivars up to growth stages 73-77, their vegetative growth was compared in terms of some selected growth parameters. For this purpose, the individual effects of the cultivars, observed at each growth stage (data pooled for other treatments at each growth stage), were considered. The reason for selecting the cultivar effect was that it was the only variable for which data was available at each growth stage. Regarding the other two main variables (ComCat and water treatments), individual effects in terms of the measured parameters were not consecutively available to encompass each selected growth stage (for instance, for CC-19 treated plants data is only measured at growth stages 34, 73-77 and at harvest).

As shown in Table 4.19, it appeared that the two cultivars were growing at more or less the same rate until they reached growth stage 34. After growth stage 34 differences between cultivars were observed for number of days to reach the subsequent growth stage and from this it appeared that the cultivar HAR-2508 developed at a relatively faster rate.

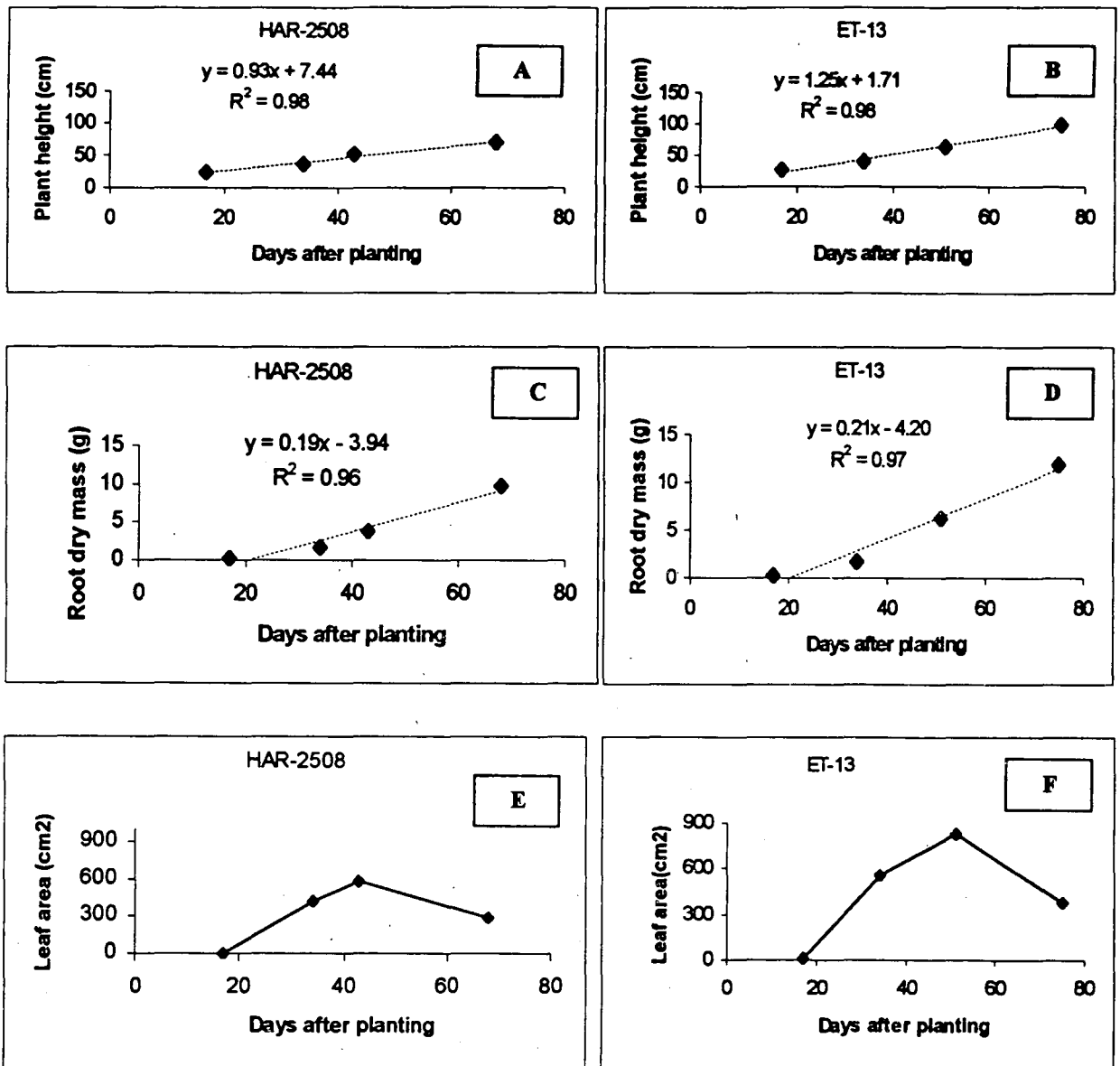
**Table 4.19:** Number of days after planting at which the cultivars reached specific growth stages.

Growth Stages	Number of days after planting for a cultivar to reach the specific growth stage	
	HAR-2508	ET-13
13	17	17
19	34	34
34	43	51
73-77	68	75

Apart from the latter observed difference, the two cultivars exhibited characteristic growth patterns. In both cultivars plant height was linearly correlated with the number of days after planting, with the  $r^2$  values for both cultivars calculated at 0.98 (Figure 4.10A & B).

Root dry mass was also found to be linearly correlated with the number of days after planting (Figure 4.10C & D), for both cultivars, with  $r^2$  values of 0.96 and 0.97 calculated for HAR-2508 and ET-13 respectively. Comparatively, ET-13 maintained a higher mean root dry mass compared to HAR-2508.

Leaf area was also linear until growth stage 34 in both cultivars, but after anthesis (i.e. following the water stress treatment), a sharp decline was observed in both cultivars (Figure 4.10E & F). Except at growth stage 13, ET-13 showed higher mean values in leaf area compared to that of HAR-2508 at each of the subsequent growth stages indicating a relatively higher rate of leaf growth for ET-13 compared to HAR-2508.



**Figure 4.10:** Characteristic growth patterns of two Ethiopian wheat cultivars as measured using different growth parameters, A) plant height of HAR-2508, B) plant height of ET-13, C) root dry mass of HAR-2508, D) root dry mass of ET-13, E) leaf area of HAR-2508 and F) leaf area of ET-13.

#### 4.4 Discussion

The vegetative growth phase of the wheat plant plays an important role in the overall yielding ability of the plant. This is partly due to the fact that the final grain yield of the crop is dependent upon the successful initiation of floral development, which occurs when the seedling has developed about 4 to 5 leaves (Clark *et al.*, 1981). Stresses during this phase of development are very likely to affect the final grain yield.

In this study attempts were made to investigate the growth performances of two Ethiopian wheat cultivars, until the early stages of grain filling, that were grown under the influences of ComCat and post anthesis water deficit stress treatments. Prior to the application of ComCat measured at growth stage 13, the two cultivars showed only slight differences in vegetative growth. At this stage the cultivar ET-13 was superior to HAR-2508 in plant height, stem thickness, stem dry mass and root dry mass, while the latter cultivar excelled in leaf area and leaf dry mass. Since both cultivars were growing without the influence of ComCat up to this growth stage, the observed differences were solely attributable to genetic differences.

Between growth stages 13 and 19 the two cultivars were grown under the influence of ComCat that was applied at growth stage 13. This ComCat application tended to enhance the growth of HAR-2508 plants in terms of plant height, stem thickness and leaf area, while the same treatment tended to inhibit the growth of ET-13 plants in terms of the same parameters. On the other hand, in terms of gain measuring parameters, ComCat application tended to inhibit growth in both cultivars, except for root dry mass in HAR-2508. Most of these responses from both cultivars were in agreement with the claims made by the manufacturers, namely that ComCat does not promote vegetative growth of above ground parts in particular, but enhances root growth (Agrorum, Germany – personal communication).

At the subsequent growth stage (34), none of the different ComCat applications (i.e. CC-13, CC-19 and CC-13 & 19) had any effect on plant height, stem thickness or leaf area in the cultivar HAR-2508. This was contrary to what was observed at

growth stage 19. However, ComCat treatment at different growth stages appeared to enhance growth in terms of mass gain parameters in both cultivars. Nevertheless, the analysis of variance revealed that all the observed differences and patterns in terms of all the growth parameters, except in root dry mass at growth stage 34, and at the three growth stages (13, 19 and 34) were all statistically non-significant at the 5% probability level. The double ComCat application (CC-13 & 19) increased the root dry mass significantly in both cultivars indicating that the enhancement of root growth by ComCat continued up till this growth stage. This tendency was more pronounced in the cultivar ET-13.

At the next growth stage (73-77), and in addition to the ComCat treatments, water stress was induced over a two week period after anthesis. None of the ComCat treatments enhanced vegetative growth in the cultivar HAR-2508 under NWS conditions. In fact, ComCat tended to inhibit vegetative growth in this cultivar, especially in terms of stem dry mass, root volume and root dry mass, under NWS conditions. The only exception was spike dry mass, where the CC-19 treatment showed a slight increase in HAR-2508 under NWS condition. However, ComCat application, particularly the double application (CC-13 & 19) tended to enhance vegetative growth in HAR-2508 plants under WS condition in terms of total leaf area, root volume, root dry mass and spike dry mass, while it had no appreciable effects on the rest of the growth measuring parameters under WS conditions.

On the other hand, the cultivar ET-13 appeared to have responded differently to the ComCat application under both conditions of water treatment. The double ComCat application (CC-13 & 19) had an increasing effect on the flag leaf area, total leaf area and stem dry mass, while the CC-13 treatment resulted in increases in both root volume and dry mass under NWS conditions, and as measured at growth stages 73-77. However, ComCat application had no appreciable effect on the other growth measuring parameters (i.e. plant height, stem thickness, leaf dry mass and spike dry mass) under NWS conditions. Under the WS condition, on the other hand, both the flag leaf and total leaf areas were increased by ComCat application at growth stage 19, while root volume and root dry mass were increased by the double

application (CC-13 & 19). In ET-13 plants grown under WS conditions, ComCat application had no appreciable effect on either plant height, stem thickness, leaf dry mass or spike dry mass. The observed enhancement of root mass in this cultivar was again in agreement with the findings of an experiment conducted under the auspices of the University of the Free State, South Africa, where the root mass of a South African wheat cultivar, SST825, was improved by the use of ComCat (Pretorius J.C.; personal communication; results not shown). Despite some substantial differences between ComCat treated and untreated plants, the analysis of variance revealed that all of these differences observed at growth stage 73-77 were non-significant at the 5% probability level. The latter indicated that conclusions regarding the effect of ComCat on vegetative growth should be treated with caution.

However, what was undoubtedly clear was that the water stress treatment caused significant and severe reductions in the vegetative growth of both cultivars, as measured in terms of many growth parameters, with the exception of plant height, stem thickness, flag leaf area and leaf dry mass. In most cases, these severe effects were alleviated by the ComCat treatments in both cultivars. Especially the CC-19 treatment tended to alleviate the reduction in total leaf area (in both cultivars), and root volume (in ET-13), while the double application (CC-13 & 19) alleviated reductions in leaf dry mass (in ET-13), root volume (in HAR-2508), root dry mass as well as spike dry mass (in both cultivars). In terms of stem dry mass, ComCat application tended to show an aggravating effect in ET-13 under WS conditions, while it not only alleviated this reduction but also increased the stem dry mass in HAR-2508. The latter alleviating effects of ComCat are in agreement with the claims made by the manufacturers (Agrorum, Germany) namely that ComCat promotes the plant's own defense mechanisms against biotic and abiotic stresses.

In order to verify the possible aggravating or alleviating effects of ComCat on the vegetative growth of the two Ethiopian wheat cultivars subjected to water deficit stress, different statistical analyses were conducted. This was also done to ascertain the possible interaction effects for the other two major factors namely the water stress treatment and possible cultivar differences. Analyses of the effects of

interaction of any two of the three main factors at a time, revealed significant interaction effects on the growth of the two Ethiopian wheat cultivars under scrutiny. At growth stages 73-77 significant water X cultivar interaction (data pooled for all ComCat treatments) were observed in terms of total leaf area, leaf dry mass and stem dry mass ( $P < 0.05$ ). In this interaction under non-water-stress conditions, ET-13 outperformed HAR-2508 in terms of the above three parameters. However, under water stress conditions, the two cultivars did not differ significantly in their leaf area indicating that ET-13 had lost more leaf area due to the stress than had HAR-2508. On the other hand, in terms of leaf dry mass, and stem dry mass the stress treatment consistently reduced the mean values obtained for both cultivars, but this did not result in a significant reduction in HAR-2508, while the opposite was true for ET-13. Considering these observations, HAR-2508 appeared to be relatively less affected by the water deficit stress. Genotype factors might have played a major role in the responses of the cultivars to the stress condition. Clark *et al.* (1981), observed differences in leaf water status between wheat genotypes and reported that such differences could stem from combinations of genetic differences in root systems and efficiency of water transport as well as from differences in leaf morphology and orientation. According to Angus and Moncur (1977) as well as Bradford and Hsiao (1982), even very mild water stress is sufficient to have substantial effects on canopy size of wheat plants, if the stress lasts for some time.

Similarly the statistical analysis of the interaction between water and ComCat treatments (data pooled for cultivars) at growth stages 73-77 revealed significant ( $P < 0.1$ ) differences only in terms of total leaf area and root dry mass. At growth stage 34, when the wheat plants were grown under well watered conditions, the double application of ComCat increased the root dry mass in ET-13 significantly. Moreover, at growth stages 73-77 the same application of ComCat increased the root dry mass (average for both cultivars) particularly under the WS conditions, confirming that the ComCat application enhanced root development, a characteristic particularly desired in crops growing under water stress conditions.

The ComCat X cultivar interaction analysis (data pooled for both water treatments) for growth stages 73-77 was also significant ( $P < 0.05$ ) in terms of leaf dry mass and spike dry mass. From this it appeared that the earliest application of (CC-13) increased the leaf dry mass in ET-13, while the double application increased the spike dry mass in the same cultivar. Changes in the dry mass of organs are difficult to interpret as beneficial or detrimental to a plant under a stress without physiological data. This aspect will be addressed more comprehensively in Chapter 6.

The statistical analysis of the individual effects of each of the three main factors also revealed some significant effects. In this regard, the analysis of cultivar differences at growth stage 19 (data pooled for water and ComCat treatments) revealed significant differences between the two cultivars in terms of stem thickness and leaf area ( $P < 0.01$ ) with HAR-2508 excelling in the former and ET-13 in the latter parameter. At the subsequent growth stage (stage 34), a significant change in the growth pattern of the cultivars was observed where the cultivar ET-13 significantly excelled ( $P < 0.01$ ) in terms of all of the measured parameters. The same tendency was also observed at growth stages 73-77. However, the lack of significant differences between cultivars at the early growth stages indicates that aging contributes to the full expression of genetic potential in these cultivars. Cultivar differences accounting for variations in growth patterns were also reported by Entz *et al.* (1992), where differences in root growth patterns were observed between different wheat cultivars that were growing in the same location. Belay *et al.* (1993) also reported significant variations in different agronomic parameters among sixty different durum wheat cultivars collected from the highlands of Ethiopia.

In considering the individual effects of ComCat, it generally appeared that the application of this biocatalytic agent contributed less to the vegetative growth of the wheat plants at growth stage 19. Most of the measurements done at growth stage 34 also revealed non-significant effects of the different ComCat treatments except in the case of root volume and root dry mass. At growth stages 73-77 there was also a significant difference among the four levels of ComCat applications in root volume. At growth stages 73-77, especially the double application of ComCat appeared to

have a considerable influence on the growth of roots, measured in terms of root dry mass. The higher dosage that the plants received when sprayed twice, as well as the fact that the second application was done when the plants had about 12 leaves, might have led to an increase in the amount of ComCat absorbed by the plant. Data obtained in this study as well as the different ways in which the data were analyzed statistically unequivocally indicated that ComCat induced root development in these two Ethiopian wheat cultivars. Moreover, ComCat treatment tended to reduce the devastating effect water deficit stress had on root development.

The third main factor, namely water, but especially the lack thereof, came into play only at and after growth stage 60 (beginning of anthesis) but resulted in significant differences in terms of several of the growth parameters as measured at growth stages 73-77, but with some exceptions. One of these exceptions was plant height. There were no significant differences in plant height between well-watered and stressed plants and this was presumably due to the fact that, by the time the water stress treatment began, the plants were already in the last phase of vegetative growth and therefore fully grown. This observation was in support of the findings of Clarke and Durley (1981) who reported that anthesis and fertilization generally mark the termination of vegetative growth in many plants of economic importance (particularly in plants with determinate growth habits). Similarly, no significant differences were found between the dry mass of spikes of well-watered and stressed plants at growth stages 73-77 indicating that the spikes somehow managed to resist the damaging effects of the water stress. Munns *et al.* (1979) as well as Barlow *et al.* (1980) reported that the apical meristems of wheat and barley are capable of surviving severe water stress by virtue of the anatomical protection afforded by the surrounding leaf sheaths and the ability of the apex to osmoregulate. McDonald and Davies (1996) as well as Saini and Westgate (2000) also reported on the ability of the developing kernels and other organs to maintain a favourable water status during water stress.

No significant difference was observed in flag leaf area between the well-watered and stressed plants at growth stages 73-77. Reports indicate that during water

stress considerable translocation of resources takes place from older tissues to actively growing organs (Larson, 1975) and water stress is known to accelerate the senescence and shedding of older leaves (Gusta and Chen, 1987). As the flag leaf is young and most active during and after anthesis, it is the last leaf to be affected by persisting water stress. The lack of significant reduction in flag leaf area, in contrast to the case with other leaves, also appeared to be due to the limited duration of the stress when the data was collected. When the water stress persisted at the subsequent stages after growth stages 73-77, the flag leaves of stressed plants were severely affected to such an extent that they were rolled and senesced prematurely. This was in agreement with Clark *et al.* (1981) who stated that if leaf water potential remains low, leaves die, starting at the base of the plant and progressing towards the top. They further stated that, under persistent drought, all but the uppermost or flag leaves may die and even the flag leaf may dry up considerably before maturity.

Contrary to the above exceptions and consistent with other reports, significant growth reduction due to the water stress was observed. Slavic (1975) reported on the sensitivity of extension growth (including leaf growth) to an internal water deficit and considered the loss of turgor as a highly probable cause. Levit (1972) also stated that the response of a plant to drought takes many forms of which the most visible is the reduction in leaf area as a consequence of fewer and smaller leaves. The significant reduction in leaf area, because of water stress, is considered as a coping strategy by the wheat plant and results from reduced growth and senescence of older leaves (Gusta and Chen, 1987).

In summary, it appeared from this study that the two Ethiopian wheat cultivars responded differently to both the water stress and the ComCat treatments, particularly at later growth stages. The cultivar ET-13 appeared to be the one most affected by the water stress. However, it must be noted that, as wheat is principally grown for its grain and not for its vegetative parts, the drawing of conclusion as to which cultivar performed better than the other under water stress can only be made when data on its yield performance is also available. This will be attended to in Chapter 5. With regard to the effect of ComCat, the biocatalyst appeared to have had a significant influence on root development but not on the growth of above ground vegetative parts. As ComCat had some effect on the dry mass content of both roots and above ground parts and in light of the difficulty to draw conclusions regarding the significance of this data, this aspect will be considered in Chapter 6 together with additional physiological data.

## Chapter 5

# Influence of cultivar differences and ComCat as well as post-anthesis water stress treatments on vegetative growth and yield of two Ethiopian wheat cultivars as measured at harvest

### 5.1 Introduction

Productivity and geographical distribution of plant species are limited as much, if not more, by water stress than by any other environmental factor (Bewley and Krochko, 1982). Water deficit stress can affect growth and development of plants at any time during the crop's life cycle. However, the extent and the nature of damage, the capacity for recovery and the impact on yield depend on the developmental stage at which the plant encounters the stress (Saini and Westgate, 2000). According to several authors (Slatyer, 1969; Hale and Orcutt, 1987; Mengel and Kirkby, 1987) the effect of drought stress on growth tends to be most pronounced in those tissues and organs which are in rapid stages of development, with primordial initiations and cell enlargement being particularly susceptible.

The reproductive phase of the wheat plant is an extremely sensitive stage in terms of the impact of water deficit stress. The importance of this stage lies in the fact that the final yield of the crop is determined by the number and size of individual kernels (Shanahan *et. al.*, 1984).

Although wheat plants growing under rain fed conditions often experience water-deficit stress, possibly at all stages of development, this study attempted to investigate the influences of such a stress during the post-anthesis period as well as the possible circumventing effect of ComCat treatments in two Ethiopian wheat cultivars. The water

deficit stress treatment is synonymously described as water stress in presentation of results and discussions.

## **5.2 Materials and Methods**

Refer to chapter 3; sections 3.1 and 3.2.

## **5.3 Results**

### **5.3.1 The effects of cultivar differences and ComCat as well as post anthesis water stress treatments on the vegetative growth and yield of two Ethiopian wheat cultivars, as measured at harvest**

#### **5.3.1.1 Vegetative growth parameters**

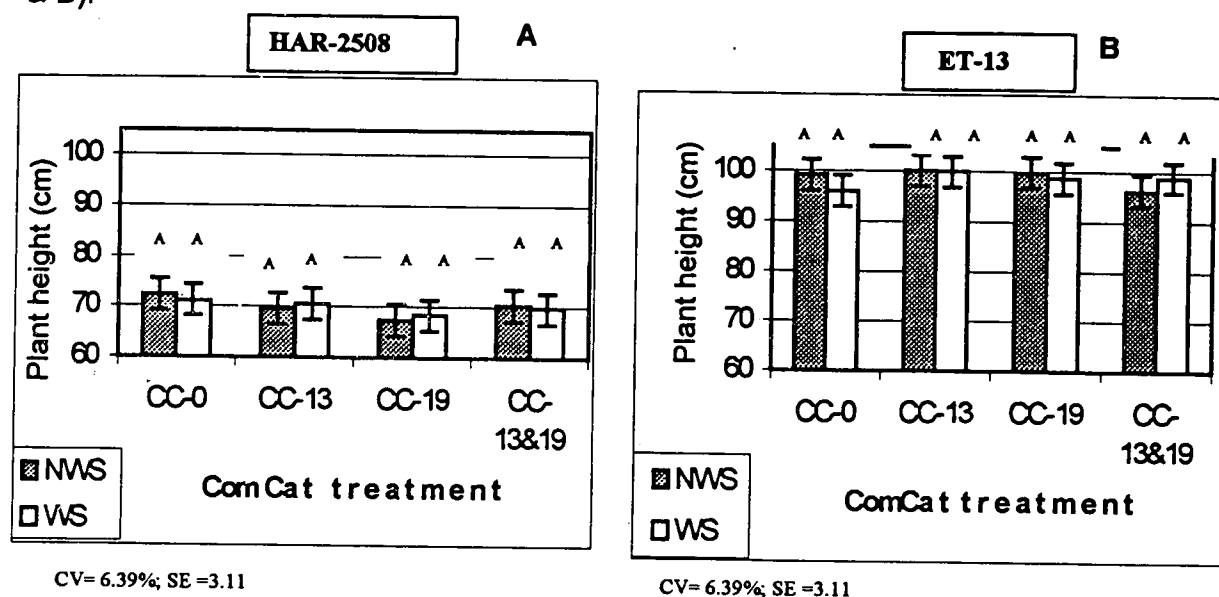
##### **Final plant height as measured at harvest**

ComCat treatment at all growth stages tended to decrease the final plant height at harvest in non-water stressed (NWS) HAR-2508 plants as compared to the untreated control (Figure 5.1A). Of the three ComCat treatments, the tallest plants were obtained with the double application of ComCat (CC-13 & 19) followed by ComCat treatment at growth stage 13 (CC-13). Similar results were obtained with HAR-2508 under water stress (WS) conditions. However, unlike the case in NWS plants, the tallest of the stressed plants were observed with the early application of ComCat (CC-13) followed by the double application (CC-13 & 19). For both normal and water deficit treatments, the application of ComCat at growth stage 19 (CC-19) resulted in the smallest HAR-2508 plants.

On the other hand, in the cultivar ET-13, it was the early application of ComCat (CC-13) that caused slightly taller plants compared to the other treatments under both levels of water treatment (Figure 5.1B). When no ComCat was applied (CC-0) water deficit stress tended to decrease the plant height slightly and this was also the case when ComCat was applied at growth stage 19. Interestingly, the double treatment of

ComCat (CC-13 & 19) tended to increase plant height while plants were stressed compared to non stressed ComCat-treated plants.

Despite the differences in plant height observed for both cultivars under the influence of different water and ComCat treatments, according to the analysis of variance, the interaction of the three main factors (cultivar, water and ComCat) was not statistically significant at the 5% probability level (as indicated by the same letters in Figures 5.1 A & B).



**Figure 5.1:** Final plant height at harvest of two Ethiopian wheat cultivars, A) HAR-2508 and B) ET-13, as influenced by cultivar differences and ComCat as well as post-anthesis water stress (WS) treatments. (Similar letters on top of the columns indicate non-significant differences between values at the 5% probability level).

### Total mass of air-dry aboveground plant parts as measured at harvest.

In non-water stressed (NWS) plants of the cultivar HAR-2508, all ComCat treatments tended to reduce the dry mass of above ground parts (Figure 5.2 A). The control NWS-plants showed 22%, 10% and 16% higher dry mass than the CC-13, CC-19 and CC-13 & 19 treated ones, respectively. However, the first ComCat treatment (CC-13) had the least effect on the total dry mass of non-stressed plants.

On the other hand, in HAR-2508 plants grown under water stress (WS) conditions, the reduction in dry mass of above ground parts was more or less similar whether ComCat was applied or not. Comparing the total mass of air-dry above ground parts of the cultivar HAR-2508 grown under NWS conditions with those grown under WS conditions revealed marked reductions in dry mass due to the WS treatment. ComCat treatment at different growth stages (CC-13, CC-19 and CC-13 & 19) of NWS plants, however, showed higher dry masses (30%, 10%, 20% and 9% respectively) than the water stressed ones. It, therefore, appeared that the application of ComCat reduced the loss of dry mass of above ground parts only under WS conditions. Particularly the double application of ComCat (CC-13 & 19) appeared to have a slight increasing effect on above ground part dry mass.

In the cultivar ET-13 grown under NWS conditions, no clear differences in dry mass were observed between the ComCat-treated and untreated plants (Figure 5.2B). However, ComCat treatment at growth stage 19 produced plants with slightly higher above ground dry mass than the control and other treatments.

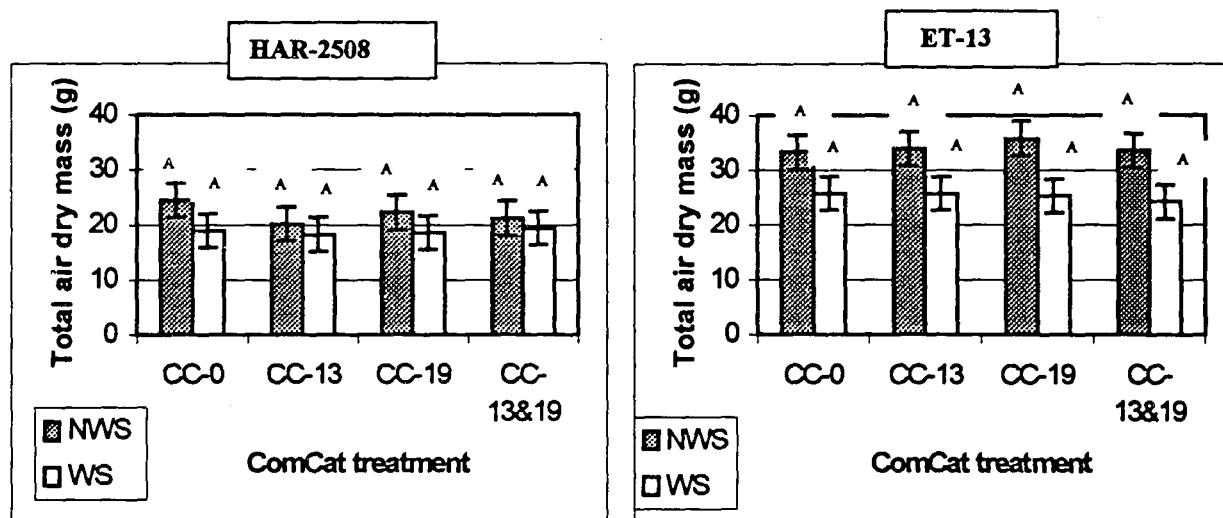


Figure 5.2: Final mass of air-dry above ground parts of two Ethiopian wheat cultivars at harvest, A) HAR-2508 and B) ET-13, as influenced by cultivar differences and ComCat (CC) as well as post-anthesis WS treatments. (Similar letters on top of the columns indicate non-significant differences between values at the 5% probability level).

The WS treatment caused a 23% reduction in dry mass of above ground parts in untreated ET-13 plants while the ComCat treatment had no alleviating or accentuating effect in this regard. Despite the differences observed in the dry mass of above ground parts of both cultivars subjected to different treatments, the analysis of variance, however, revealed all differences to be non-significant at the 5% probability level (Fig. 5.2A & B). This is understandable when non-stressed as well as stressed plants of the same cultivar are compared together. However, when non-stressed plants of the same cultivar were compared to stressed plants regarding the dry mass of above ground parts under the influence of the ComCat treatment at different growth stages, considerable differences were observed. For instance, in ET-13 plants, differences of 29%, 32%, 41% and 38% for untreated control, CC-13, CC-19 and CC-13 & 19 treatments, respectively, were observed. These differences seem to be important despite the outcome of the statistical analysis procedure.

### 5.3.1.2 Yield components

#### Average number of kernels per spike

**Table 5.1:** Influence of water stress as well as ComCat treatments on number of kernels per spike in two Ethiopian wheat cultivars.

Cultivars and ComCat treatment	Number of kernels per spike	
	NWS condition	WS condition
HAR-2508 X CC-0	30.03 ± 3.27 A	31.90 ± 16.01 A
HAR-2508 X CC-13	31.57 ± 3.92 A	28.20 ± 8.49 A
HAR-2508 X CC-19	28.37 ± 3.97 A	29.97 ± 8.36 A
HAR-2508 X CC-13 & 19	29.23 ± 1.27 A	31.10 ± 5.14 A
ET-13 X CC-0	42.80 ± 3.42 A	38.03 ± 3.42 A
ET-13 X CC-13	44.40 ± 1.61 A	38.00 ± 4.00 A
ET-13 X CC-19	46.13 ± 6.47 A	34.62 ± 3.36 A
ET-13 X CC-13 & 19	41.57 ± 2.14 A	37.70 ± 7.08 A

Coefficient of variation (CV)= 13.97%. Standard error (SE) = 2.8412. Similar letters indicate non-significant differences at the 5% probability level. ± = Standard deviation for three replications.

The average number of kernels per spike in the cultivar HAR-2508 grown under NWS conditions was slightly higher in plants that received ComCat at growth stage 13, compared to the other treatments (Table 5.1). On the other hand, under WS, ComCat application did not have an appreciable effect on the number of kernels per spike in the same cultivar.

In ET-13 plants grown under NWS conditions, the application of ComCat at growth stage 19 increased the number of kernels per spike by 8% compared to the control. ComCat treatment at growth stage 13 (CC-13) also increased the number of kernels slightly while the double application treatment (CC-13 & 19) reduced the number of kernels per spike. On the other hand, the WS treatment reduced the number of kernels per spike in all differently treated ET-13 plants (Table 5.1) while the double ComCat treatment (CC-13 & 19) actually accentuated this reduction.

In general, the water stress treatment caused considerable reduction in the average number of kernels per spike in ET-13 plants but this was not the case in HAR-2508 plants (Table 5.1). The control CC-13, CC-19 and CC-13 & 19 treated ET-13 plants that grew under NWS conditions had 13%, 17%, 33% and 10% higher average number of kernels per spike, respectively, compared to their counterparts grown under WS conditions. It, therefore, appeared that the cultivar ET-13 was more drought sensitive than HAR-2508. However, the analysis of variance revealed that all of the observed differences were statistically non-significant at the 5% probability level.

### Total number of kernels per three plants (grown in one pot)

**Table 5.2:** Influence of water stress as well as ComCat treatments on the total number of kernels per three plants (in a pot) of two Ethiopian wheat cultivars.

Cultivars and ComCat treatment	Total number of kernels per three plants (in a pot)	
	NWS condition	WS condition
HAR-2508 X CC-0	240.00 ± 37.47 A	223.33 ± 16.01 A
HAR-2508 X CC-13	189.33 ± 23.71 A	169.00 ± 55.43 A
HAR-2508 X CC-19	237.00 ± 42.58 A	197.33 ± 42.91 A
HAR-2508 X CC-13 & 19	204.67 ± 8.62 A	215.33 ± 27.30 A
ET-13 X CC-0	385.33 ± 31.13 A	328.67 ± 20.01 A
ET-13 X CC-13	369.33 ± 13.65 A	353.00 ± 16.82 A
ET-13 X CC-19	379.33 ± 40.67 A	309.67 ± 4.51 A
ET-13 X CC-13 & 19	374.00 ± 19.31 A	334.67 ± 25.54 A

Coefficient of variation (CV) = 10.86%. Standard error (SE) = 17.6689. Similar letters indicate non-significant differences at the 5% probability level. ± = Standard deviation for three replications.

The application of ComCat in plants of the cultivar HAR-2508 grown under both NWS and WS conditions tended to reduce the total number of kernels. Particularly the early application of ComCat resulted in the highest reduction in the number of kernels under both water treatment conditions (Table 5.2). Moreover, WS reduced the total number of kernels in the same cultivar quite severely while the double ComCat treatment (CC-13 & 19) limited this reduction to the extent that the water stressed plants performed better than the non-stressed control in this regard.

All ComCat applications also tended to reduce the total number of kernels in the cultivar ET-13 grown under NWS conditions (Table 5.2). However, under WS both the early (CC-13) and the double (CC-13 & 19) application of ComCat resulted in the least reduction in the total number of kernels, compared to non-stressed plants.

The statistical analysis, however, did not reveal any significant differences among the mean values at the 5% probability level.

### Fresh mass of air-dry kernels (total yield) for three plants

**Table 5.3:** Influence of water stress as well as ComCat treatments on the fresh mass of air-dry kernels (total yield) for three plants (in a pot) of two Ethiopian wheat cultivars.

Cultivars and ComCat treatment	Fresh mass of air-dry kernels (g)	
	NWS condition	WS condition
HAR-2508 X CC-0	10.88 ± 0.92 A	8.16 ± 0.64 A
HAR-2508 X CC-13	8.78 ± 1.16 A	7.29 ± 2.15 A
HAR-2508 X CC-19	9.77 ± 0.44 A	7.63 ± 0.87 A
HAR-2508 X CC-13 & 19	9.37 ± 0.84 A	8.21 ± 1.40 A
ET-13 X CC-0	13.44 ± 0.38 A	9.28 ± 1.27 A
ET-13 X CC-13	13.40 ± 1.55 A	9.43 ± 0.91 A
ET-13 X CC-19	13.96 ± 1.53 A	9.23 ± 1.45 A
ET-13 X CC-13 & 19	13.48 ± 1.42 A	9.21 ± 1.57 A

Coefficient of variation (CV) = 9.08%. Standard error (SE) = 0.5292. Similar letters indicate non-significant differences at the 5% probability level. ± = Standard deviation for three replications.

Application of ComCat tended to reduce the fresh mass of kernels in the cultivar HAR-2508 under both conditions of water treatment (Table 5.3). Particularly the early

application of ComCat showed the same reducing tendency as was observed for total number of kernels per three plants (Table 5.2). The double ComCat treatment (CC-13 & 19), however, again tended to minimize this reduction process.

In the cultivar ET-13, ComCat application appeared to have had no appreciable effect on the fresh mass of kernels under both conditions of water treatment (Table 5.3). On the other hand, the fresh mass of kernels was considerably reduced in both cultivars under WS conditions. Moreover, this reduction in the air-dry mass of kernels, due to water stress, was much higher in the cultivar ET-13 than that in HAR-2508. In the former cultivar, reductions of 31%, 30%, 34% and 32% were observed in the control as well as in the CC-13, CC-19 and CC-13 & 19 ComCat treated plants, respectively, under the WS condition as compared to that of the same treatments under the NWS condition (Table 5.3). The ComCat treatments did not alleviate or accentuate these reductions in ET-13. Despite these substantial differences, however, the analysis of variance once again revealed them to be non-significant at the 5% probability level.

#### **Hundred kernel mass**

Treating HAR-2508 plants with ComCat, particularly at growth stages 13, tended to increase the hundred kernel mass under both levels of water treatment, while the effect was more pronounced under the WS condition (Table 5.4). Under the WS condition, each of the ComCat treatments in the same cultivar resulted in higher hundred kernel mass compared to that of the untreated control. Treatment with ComCat in ET-13 plants also showed a similar increasing tendency in hundred kernel mass under both levels of water treatment. The CC-13 and CC-19 treatments, in the same cultivar, resulted in the highest hundred kernel masses compared to the untreated controls as well as the other ComCat treatments under NWS and WS conditions respectively (Table 5.4).

**Table 5.4:** Influence of water stress as well as ComCat treatments on the hundred kernel mass of two Ethiopian wheat cultivars.

Cultivars and ComCat treatment	Hundred kernel mass (g)	
	NWS condition	WS condition
HAR-2508 X CC-0	4.63 ± 0.29 A	3.62 ± 0.54 A
HAR-2508 X CC-13	4.69 ± 0.22 A	4.31 ± 0.27 A
HAR-2508 X CC-19	4.32 ± 0.71 A	3.94 ± 0.75 A
HAR-2508 X CC-13 & 19	4.58 ± 0.61 A	3.84 ± 0.45 A
ET-13 X CC-0	3.73 ± 0.29 A	2.77 ± 0.34 A
ET-13 X CC-13	4.00 ± 0.26 A	2.69 ± 0.17 A
ET-13 X CC-19	3.85 ± 0.15 A	3.05 ± 0.39 A
ET-13 X CC-13 & 19	3.79 ± 0.36 A	2.86 ± 0.35 A

Coefficient of variation (CV) = 7.89%. Standard error (SE) = 0.1726. Similar letters indicate non-significant difference at the 5% probability level. ±, Standard deviation for three replications.

As was observed with the other yield parameters, water stress caused a severe reduction in the hundred kernel mass in both cultivars. However, this reduction tended to be substantially alleviated by ComCat treatment in both cultivars. The reduction in hundred kernel mass due to the water stress in the cultivar HAR-2508 was 22% and 8% in the untreated and CC-13 treated plants respectively. In fact, all the other ComCat treatments also had alleviating effects in the same cultivar. Similarly, the reduction in hundred kernel mass in the cultivar ET-13 was 26% and 21% in the

untreated control and CC-19 treated plants, respectively. However, the early application of ComCat (CC-13) had an accentuating effect in the same cultivar.

Once again, none of the observed differences were statistically significant according to the analysis of variance procedure at the 5% probability level.

### Harvest index (HI)

**Table 5.5:** Influence of water stress as well as ComCat treatments on the harvest index of two Ethiopian wheat cultivars.

Cultivars and ComCat treatment	Harvest index	
	NWS condition	WS condition
HAR-2508 X CC-0	0.44 ± 0.01 A	0.43 ± 0.02 A
HAR-2508 X CC-13	0.44 ± 0.02 A	0.40 ± 0.04 A
HAR-2508 X CC-19	0.44 ± 0.02 A	0.41 ± 0.02 A
HAR-2508 X CC-13 & 19	0.44 ± 0.02 A	0.42 ± 0.03 A
ET-13 X CC-0	0.40 ± 0.02 A	0.36 ± 0.03 A
ET-13 X CC-13	0.40 ± 0.02 A	0.37 ± 0.03 A
ET-13 X CC-19	0.39 ± 0.01 A	0.36 ± 0.04 A
ET-13 X CC-13 & 19	0.40 ± 0.01 A	0.39 ± 0.04 A

Coefficient of variation (CV) = 5.28%. Standard error (SE) = 0.00124. Similar letters indicate non-significant differences at the 5% probability level. ± = Standard deviation for three replications.

No noticeable variation in harvest index was observed in both cultivars for all the ComCat treatments under the NWS condition (Table 5.5). However, in water stressed ET-13 plants a slight reduction in (HI) was observed while ComCat treatments had no alleviating or accentuating effects. Again, these reductions were statistically non-significant at the 5% probability level

### **5.3.2 Significance test for the correlation among the vegetative growth and yield parameters**

In order to investigate the possible relationships between vegetative growth and yield components, a correlation analysis was conducted (values pooled over three replications for each treatment combination involving the three main factors; Table 5.6). According to this statistical analysis procedure, the most essential yield component, i.e. yield per unit area, was strongly and positively associated ( $P < 0.01$ ) with all parameters measured, except for 100 kernel mass and harvest index. The number of kernels per spike also strongly and positively correlated ( $P < 0.01$ ) with all parameters, except for the harvest index. The total number of kernels was strongly associated ( $P < 0.01$ ) with all parameters measured.

Similarly, plant height strongly and positively correlated with most of the measured parameters except for negative correlation with hundred kernel mass and harvest index. The total number of kernels strongly but negatively correlated ( $P < 0.01$ ) with hundred kernel mass and harvest index. It positively correlated with plant height, and mass of air-dry above ground parts as well as all the other yield components.

**Table 5.6:** Correlation coefficients for all possible combinations of vegetative growth parameters and yield components of wheat.

	Height	Number of kernels spike <sup>-1</sup>	Fresh mass of air-dry kernels	Total number of kernels per three plants	Hundred kernel mass	Grain yield	Total fresh mass of air-dry above ground parts
Number of kernels spike <sup>-1</sup>	0.76**						
Fresh mass of air-dry kernels	0.56**	0.73**					
Total number of kernels per three plants	0.88**	0.86**	0.76**				
Hundred kernel mass	-0.58**	-0.39**	0.10 <sup>ns</sup>	-0.55**			
Grain yield	0.56**	0.73**	1.00**	0.76**	0.10 <sup>ns</sup>		
Total fresh mass of air-dry above ground parts	0.75*	0.79**	0.95**	0.87**	-0.11 <sup>ns</sup>	0.95**	
Harvest index	-0.65**	-0.21 <sup>ns</sup>	0.07 <sup>ns</sup>	-0.40**	0.63**	0.07 <sup>ns</sup>	-0.24 <sup>ns</sup>

\*, \*\* = Significant correlation at 5% and 1 % probability levels respectively. ns = non-significant correlation at the 5 % probability level.

### 5.3.3 Statistical analysis of cultivar X ComCat treatment interaction in terms of vegetative and yield parameters in two Ethiopian wheat cultivars.

#### 5.3.3.1 Interaction in terms of vegetative parameters

**Table 5.7:** Statistical analysis of cultivar X ComCat treatment interaction in terms of vegetative growth parameters in two Ethiopian wheat cultivars.

Treatment combinations (cultivars x ComCat)	Plant height (cm)	Mass of air-dry above ground parts (g)
HAR-2508 X CC -0	71.77 <sup>A</sup>	21.70 <sup>A</sup>
HAR-2508 X CC -13	70.08 <sup>A</sup>	19.16 <sup>A</sup>
HAR-2508 X CC -19	67.92 <sup>A</sup>	20.43 <sup>A</sup>
HAR-2508 X CC -13 & 19	70.13 <sup>A</sup>	20.24 <sup>A</sup>
ET-13 X CC -0	97.60 <sup>A</sup>	29.51 <sup>A</sup>
ET-13 X CC -13	100.10 <sup>A</sup>	29.84 <sup>A</sup>
ET-13 X CC -19	99.32 <sup>A</sup>	30.54 <sup>A</sup>
ET-13 X CC -13 & 19	97.60 <sup>A</sup>	28.93 <sup>A</sup>
<b>CV (%)</b>	6.39	7.93
<b>SE</b>	2.1991	0.811

Similar letters indicate non-significant differences at the 5% probability level.

Statistical analysis of the cultivar X ComCat treatment interaction for HAR-2508 (values pooled for both levels of water treatment) showed that the ComCat application did not have a considerable effect on the final plant height in this cultivar (Table 5.7). The average height of all HAR-2508 control plants (i.e. no ComCat application) grown

under both levels of water treatment did not differ significantly from plants treated with ComCat at different growth stages. On the other hand, the early application of ComCat to ET-13 plants at growth stage 13 appeared to have a slight increasing effect on the final plant height (Table 5.7). However, neither this effect nor those of the later ComCat treatments were statistically significant at the 5% probability level. Similar statistically non-significant differences in effects were observed for both cultivars in terms of the effect of ComCat treatment on the mass of air-dry above ground parts under both levels of water treatment (Table 5.7).

### 5.3.3.2 Interaction in terms of yield parameters

**Table 5.8:** Statistical analysis of cultivar X ComCat treatment interaction in terms of yield parameters in two Ethiopian wheat cultivars.

Interaction (cultivars x ComCat)	Number of kernels per spike	Total number of kernels per three plants ( $\phi$ )	Fresh mass of air-dry kernels per three plants (g)	Hundred kernel mass (g)	Grain yield (g m <sup>-2</sup> )	Harvest index
HAR-2508 X CC-0	30.97 <sub>A</sub>	231.67 <sub>B</sub>	9.52 <sub>A</sub>	4.13 <sub>A</sub>	445.06 <sub>A</sub>	0.44 <sub>A</sub>
HAR-2508 X CC -13	29.88 <sub>A</sub>	179.17 <sub>C</sub>	8.03 <sub>A</sub>	4.50 <sub>A</sub>	375.23 <sub>A</sub>	0.42 <sub>A</sub>
HAR-2508 X CC -19	29.17 <sub>A</sub>	217.17 <sub>B</sub>	8.70 <sub>A</sub>	4.13 <sub>A</sub>	406.48 <sub>A</sub>	0.42 <sub>A</sub>
HAR-2508 X CC -13 & 19	30.17 <sub>A</sub>	210.00 <sub>B</sub>	8.79 <sub>A</sub>	4.21 <sub>A</sub>	410.71 <sub>A</sub>	0.43 <sub>A</sub>
ET-13 X CC -0	40.42 <sub>A</sub>	357.00 <sub>A</sub>	11.36 <sub>A</sub>	3.25 <sub>A</sub>	530.84 <sub>A</sub>	0.38 <sub>A</sub>
ET-13 X CC -13	41.20 <sub>A</sub>	361.17 <sub>A</sub>	11.42 <sub>A</sub>	3.34 <sub>A</sub>	533.49 <sub>A</sub>	0.38 <sub>A</sub>
ET-13 X CC -19	40.38 <sub>A</sub>	344.50 <sub>A</sub>	11.59 <sub>A</sub>	3.45 <sub>A</sub>	541.64 <sub>A</sub>	0.38 <sub>A</sub>
ET-13 X CC -13 & 19	39.63 <sub>A</sub>	354.33 <sub>A</sub>	11.35 <sub>A</sub>	3.33 <sub>A</sub>	530.13 <sub>A</sub>	0.39 <sub>A</sub>
<b>CV (%)</b>	13.97	10.86	9.08	7.89	9.08	5.28
<b>SE</b>	2.009	12.4938	0.3742	0.1221	17.4852	0.0087

$\phi$  = (mean separation was done using Duncan's multiple range test at the 10% probability level). Similar letters indicate non-significant differences, while different letters indicate otherwise at the 10% probability level.

According to the analysis of variance, the interaction between cultivar and ComCat (values pooled for both levels of water treatment) was only significant for total number of kernels ( $P < 0.1$ ; Table 5.8) and differences between ComCat treatments were significant only for the cultivar HAR-2508. The average number of kernels of HAR-2508 plants that received the earliest application of ComCat (CC-13) was the least as compared to that of the other ComCat treatments, including the untreated controls (Table 5.8). The highest number of kernels, in the cultivar HAR-2508, were observed in the untreated control plants, which was 29%, 7% and 10% higher than that of CC-13, CC-19 and CC-13 & 19 treated plants, respectively. On the other hand, the early (CC-13) application of ComCat appeared to have a slight increasing effect on the number of kernels in the cultivar ET-13, but the difference was not statistically significant at the 5% probability level.

Despite the non-significant interaction effect calculated for all of the remaining yield parameters, some differences were observed which deserve mentioning. In HAR-2508, the cultivar X CC-13 treatment interaction value calculated for hundred kernel mass was the highest indicating that this treatment improved the dry mass of kernels. Improvement by the early ComCat treatment (CC-13) was not only in the hundred kernel mass, but also in most of the other yield parameters and this was statistically confirmed for the cultivar ET-13. Cultivar X ComCat treatment interaction calculations also showed that treatment with ComCat at growth stage 19 improved the mass of air-dry kernels, hundred kernel mass and total grain yield, albeit not to a statistically significant degree.

### 5.3.4 Statistical analysis of water X ComCat treatment interaction in terms of vegetative growth and yield parameters in two Ethiopian wheat cultivars.

#### 5.3.4.1 Interaction in terms of vegetative parameters

The application of ComCat to plants (pooled values obtained for both cultivars) did not have an appreciable effect on plant height under both levels of water treatment (Table 5.9). Similarly, no considerable effect of ComCat application on mass of air-dry above ground parts were observed. The analysis of variance confirmed the non-significance of these differences for both levels of water treatment and for both vegetative growth parameters (Table 5.9).

**Table 5.9:** Statistical analysis of water X ComCat treatment interaction in terms of vegetative growth parameters in two Ethiopian wheat cultivars.

Interaction (Water x ComCat)	Plant height (cm)	Mass of air-dry above ground parts (g)
Non WS X CC-0	85.80 <sup>A</sup>	28.92 <sup>A</sup>
Non WS X CC-13	84.95 <sup>A</sup>	27.03 <sup>A</sup>
Non WS X CC-19	83.65 <sup>A</sup>	29.03 <sup>A</sup>
Non WS X CC-13 & 19	83.35 <sup>A</sup>	27.38 <sup>A</sup>
WS X CC-0	83.57 <sup>A</sup>	22.29 <sup>A</sup>
WS X CC-13	85.27 <sup>A</sup>	21.97 <sup>A</sup>
WS X CC-19	83.58 <sup>A</sup>	21.94 <sup>A</sup>
WS X CC-13 & 19	84.38 <sup>A</sup>	21.80 <sup>A</sup>
C.V (%)	6.39	7.93
SE	2.1991	0.811

Similar letters indicate non-significant differences among the means at the 5% probability level.

### 5.3.4.2 Interaction in terms of yield parameters

The water X ComCat treatment interaction under NWS conditions gave non-consistent results in terms of different yield parameters. The values of control plants (pooled values obtained for both cultivars) were higher in terms of total number of kernels, fresh mass of air-dry kernels, grain yield per unit area and harvest index than those of plants grown under the same conditions but received ComCat at different growth stages (Table 5.10).

**Table 5.10:** Statistical analysis of water X ComCat treatment interaction in terms of yield parameters in two Ethiopian wheat cultivars.

Interaction (water x CC)	Number of kernels per spike	Total number of kernels per three plants	Fresh mass of air-dry kernels per three plants (g)	Hundred kernel mass (g)	Grain yield (g m <sup>-2</sup> )	Harvest index
Non WS X CC-0	36.42 <sub>A</sub>	312.67 <sub>A</sub>	12.16 <sub>A</sub>	4.18 <sub>A</sub>	568.28 <sub>A</sub>	0.424 <sub>A</sub>
Non WS X CC-13	37.98 <sub>A</sub>	279.33 <sub>A</sub>	11.09 <sub>A</sub>	4.34 <sub>A</sub>	518.32 <sub>A</sub>	0.416 <sub>A</sub>
Non WS X CC-19	37.25 <sub>A</sub>	308.17 <sub>A</sub>	11.86 <sub>A</sub>	4.08 <sub>A</sub>	554.31 <sub>A</sub>	0.415 <sub>A</sub>
Non WS X CC-13 & 19	35.40 <sub>A</sub>	289.33 <sub>A</sub>	11.43 <sub>A</sub>	4.18 <sub>A</sub>	533.98 <sub>A</sub>	0.423 <sub>A</sub>
WS X CC-0	34.97 <sub>A</sub>	276.00 <sub>A</sub>	8.72 <sub>A</sub>	3.20 <sub>A</sub>	407.62 <sub>A</sub>	0.396 <sub>A</sub>
WS X CC-13	33.10 <sub>A</sub>	261.00 <sub>A</sub>	8.36 <sub>A</sub>	3.50 <sub>A</sub>	390.40 <sub>A</sub>	0.380 <sub>A</sub>
WS X CC-19	32.30 <sub>A</sub>	253.50 <sub>A</sub>	8.43 <sub>A</sub>	3.49 <sub>A</sub>	393.82 <sub>A</sub>	0.387 <sub>A</sub>
WS X CC-13 & 19	34.40 <sub>A</sub>	275.00 <sub>A</sub>	8.71 <sub>A</sub>	3.35 <sub>A</sub>	406.86 <sub>A</sub>	0.400 <sub>A</sub>
C.V (%)	13.97	10.86	9.08	7.89	9.08	5.28
SE	2.009	12.4938	0.3742	0.1221	17.4852	0.0087

Similar letters indicate non-significant differences among the means at the 5% probability level.

On the other hand, the values of CC-13 treated plants were higher than that of each of the other ComCat treatments in terms of both number of kernels per spike and hundred kernel mass.

Under WS conditions, similar non-consistent results were obtained. Treatment with ComCat under WS conditions had a slight improving effect only with regard to hundred kernel mass (CC-13) and harvest index (CC-13 & 19) (Table 5.10). Values measured for all the other yield parameters decreased as a result of the ComCat treatments at different growth stages as compared to the untreated controls. However, according to the analysis of variance, the interaction of the two factors was non-significant at the 5% probability level.

### **5.3.5 Statistical analysis of cultivar X water treatment interaction in terms of vegetative and yield parameters in two Ethiopian wheat cultivars**

#### **5.3.5.1 Interaction in terms of vegetative growth parameters**

Statistically, the water stress treatment had no significant effect on plant height but caused a significant reduction in the mass of air-dry above ground parts of 15% and 26% in HAR-2508 and ET-13 respectively (Table 5.11).

**Table 5.11:** Statistical analysis of cultivar X water treatment interaction in terms of vegetative growth parameters in two Ethiopian wheat cultivars.

Interaction (water X cultivar)	Plant height (cm)		Mass of air-dry above ground parts (g)	
	HAR-2508	ET-13	HAR-2508	ET-13
NWS condition	69.95 <sup>A</sup>	98.91 <sup>A</sup>	22.03 <sup>C</sup>	34.14 <sup>A</sup>
WS condition	70.00 <sup>A</sup>	98.40 <sup>A</sup>	18.74 <sup>D</sup>	25.27 <sup>B</sup>
CV (%)	6.39		7.93	
SE	1.555		0.5735	

Different letters indicate significant difference ( $P < 0.01$ ), while similar letters indicate non-significant differences among the means at the 5% probability level.

### 5.3.5.2 Interaction in terms of yield parameters

Except for harvest index, the WS treatment caused significant reductions in all the other yield parameters in both HAR-2508 and ET-13 (Table 5.12). These reductions, particularly in the cultivar ET-13, were consistently significant for all the measured parameters while no statistically significant reductions were caused by the water stress treatment in the cultivar HAR-2508 in terms of number of kernels per spike and total number of kernels.

**Table 5.12:** Statistical analysis of cultivar X water treatment interaction in terms of yield parameters in two Ethiopian wheat cultivars.

Cultivars	Water treatment	Number of kernels per spike	Total number of kernels per three plants $\delta$	Fresh mass of air-dry kernels per three plants (g)	Hundred kernel mass (g)	Grain yield ( $\text{g m}^{-2}$ )	Harvest index
HAR-2508	Non water stress	29.80 c	217.75 c	9.70 B	4.55 A	453.35 B	0.44 A
	Water stress	30.29 c	201.25 c	7.82 c	3.93 B	365.39 c	0.42 A
ET-13	Non water stress	43.73 A	377.00 A	13.57 A	3.84 B	634.07 A	0.40 A
	Water stress	37.09 B	331.50 B	9.29 B	2.84 c	433.96 B	0.37 A
CV (%)		13.97	10.86	9.08	7.89	9.08	5.28
SE		1.4206	8.8345	0.2646	0.0863	12.3639	0.0062

$\delta$  = Significant difference at the 10 % probability level in the LSD procedure.

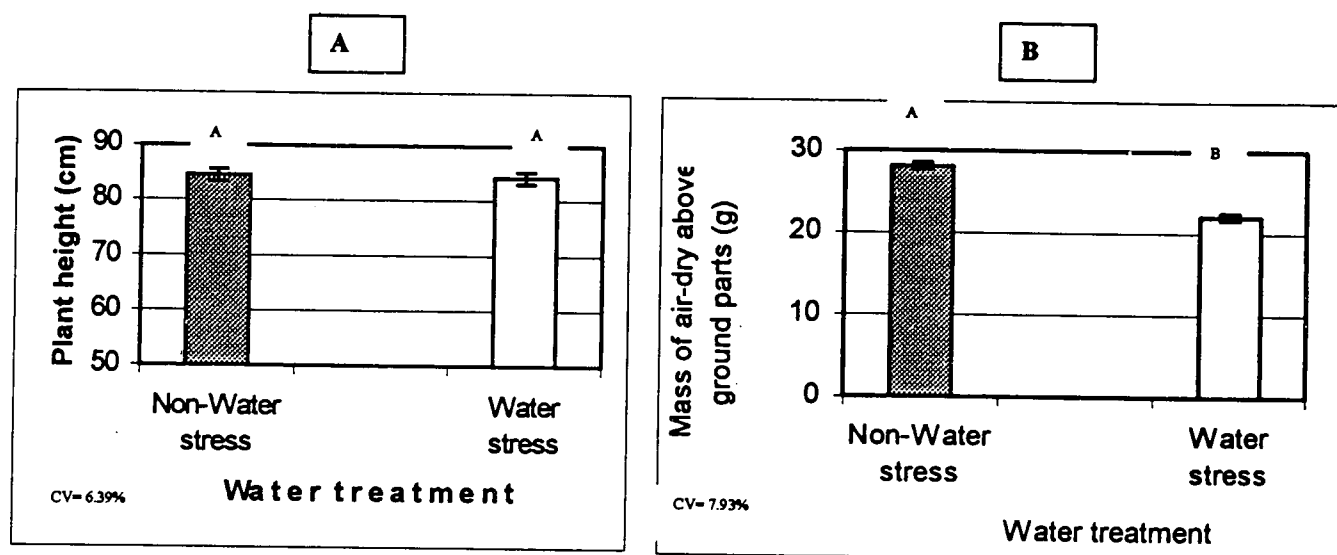
Different letters indicate significant difference between mean values, while similar letters indicate otherwise at the 5% probability level.

Significant reductions in fresh mass of air-dry kernels (-19%), hundred kernel mass (-14%) and grain yield per unit area (-19%) were observed in HAR-2508. In the cultivar ET-13 the effect of the water stress treatment was more pronounced and caused reductions in the number of kernels per spike (-15%), total number of kernels (-12%), fresh mass of air-dry kernels (-32%), hundred kernel mass (-26%) and grain yield per unit area (-32%) (Table 5.12).

### 5.3.6 Statistical analysis of individual effects of cultivar differences as well as water and ComCat treatments in terms of vegetative and yield parameters in two Ethiopian wheat cultivars.

Following the statistical analysis of the influences of all possible combinations of the three main factors on different parameters, the individual effects of each of these factors were also statistically analyzed.

#### 5.3.6.1 Individual effects of the water treatment



**Figure 5.3:** Individual effect of water treatment on A) plant height and B) on mass of air-dry above ground plant parts in two Ethiopian wheat cultivars (values pooled for cultivars and ComCat levels). Different letters indicate significant difference between the two water levels at the 1% probability level, while similar letters indicate non-significant difference at the 5% probability level.

The analysis of variance revealed statistically significant differences ( $P < 0.001$ ) between the two levels of water treatment in terms of all of the measured parameters except for plant height (Figure 5.3A; values pooled for both cultivars and for ComCat treatments). Mean values of pooled data for both cultivars and ComCat treatments

revealed statistically significant differences in the mass of air-dry above ground parts between the two levels of water treatment (Figure 5.3 B). The water stress treatment clearly reduced the mass of above ground parts.

Statistically (pooled data for both cultivars and for all ComCat treatments), the individual water stress effect on all the measured yield parameters was significant (Table 5.13). In all cases water stress caused a reduction in measured yield parameter values and this was most severe for air dry mass of kernels (-27%), hundred kernel mass (-19%) and grain yield per unit area (-26%).

**Table 5.13:** Statistical analyses of individual effects of water treatment in terms of yield parameters in two Ethiopian wheat cultivars

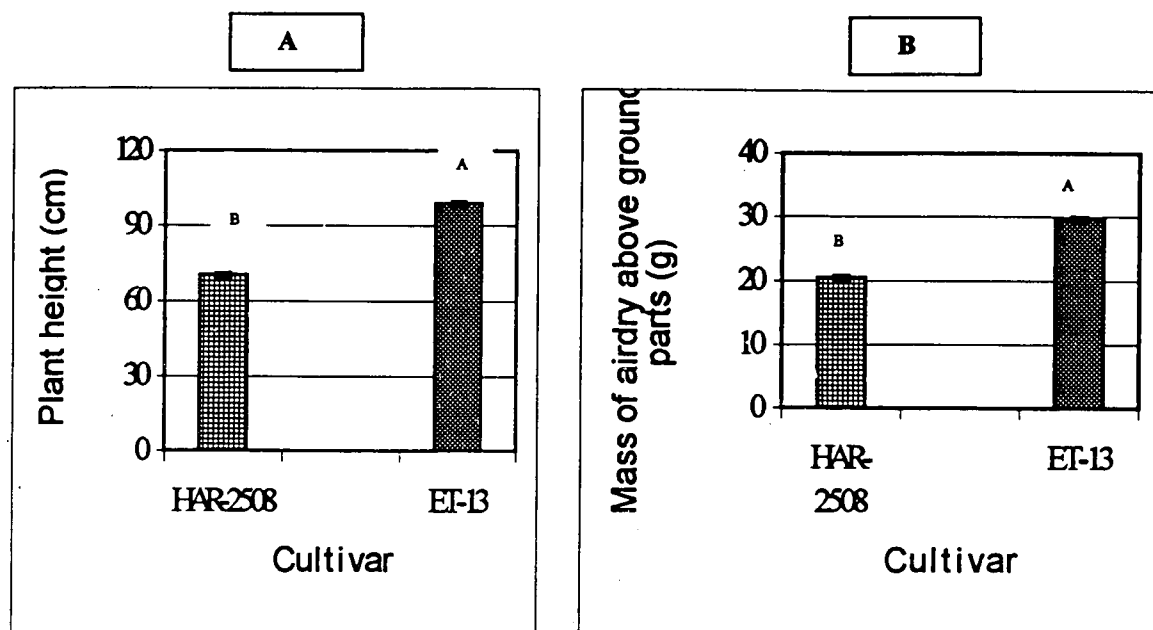
Levels of water treatment	Number of kernels per spike $\beta$	Total number of kernels per three plants	Fresh Mass of air-dry kernels per three plants (g)	Hundred kernel mass (g)	Grain yield per unit area (g m <sup>-2</sup> )	Harvest index
NWS	36.76 A	297.38 A	11.64 A	4.20 A	543.72 A	0.42 A
WS	33.69 B	266.38 B	8.55 B	3.39 B	399.67 B	0.39 B
C.V %	13.97	10.86	9.08	7.89	9.08	5.28
SE	1.0045	6.2469	0.1871	0.0610	8.7426	0.0044

$\beta$  = mean separation was performed using LSD at the 5% probability level, while in the other parameters different letters indicate significant difference at the 1% probability level,

### 5.3.6.2 Cultivar difference effects

According to the analysis of variance, significant differences were observed between the two cultivars for both vegetative growth parameters (i.e. plant height, Fig. 5.4A, and mass of air-dry above ground parts, Fig. 5.4B) as well as for yield parameters (Table 5.14). The cultivar ET-13 was clearly the taller grower and outperformed HAR-2508 with regard to plant height (+41%) and total fresh mass of above ground parts (+46%) (Figures 5.4A and B). Moreover, ET-13 also measured higher than HAR-2508 with regard to number of kernels per spike (+34%), total number of kernels (+69%),

fresh mass of air-dry kernels (+30%), and yield per unit area (+30%). HAR-2508 only measured higher in hundred kernel mass (+27%) and harvest index (+12%) (Table 5.14).



**Figure 5.4:** Individual effects of cultivar differences on vegetative growth parameters as measured at harvest (values pooled for water and ComCat levels). The different letters indicate significant difference between the two cultivars at the 1 % probability level.

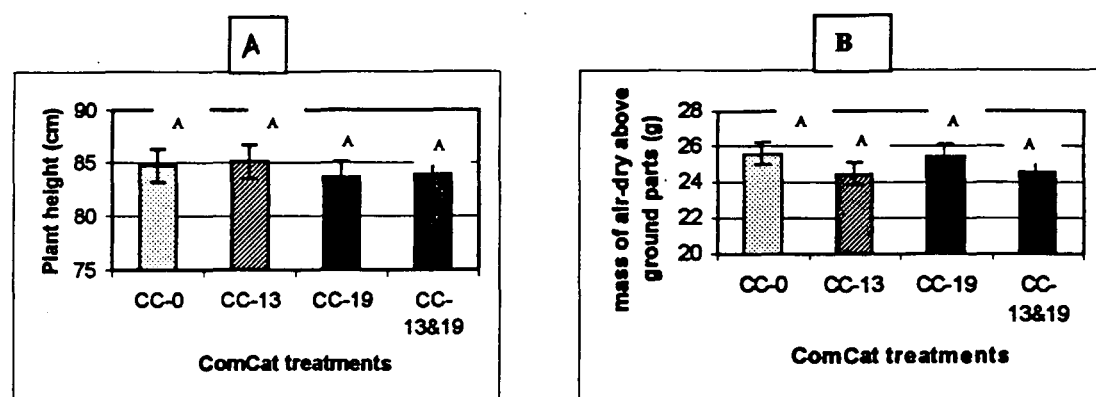
**Table 5.14:** Statistical analysis of individual effects of cultivar differences on yield components of two Ethiopian wheat cultivars.

Cultivars	Number of kernels per spike	Total Number of kernels per three plants	Fresh Mass of air-dry kernels per three plants (g)	Hundred kernel mass (g)	Grain yield per unit area ( $\text{g m}^{-2}$ )	Harvest index
<b>HAR-2508</b>	30.05 <sub>B</sub>	209.50 <sub>B</sub>	8.76 <sub>B</sub>	4.24 <sub>A</sub>	409.37 <sub>B</sub>	0.43 <sub>A</sub>
<b>ET-13</b>	40.41 <sub>A</sub>	354.25 <sub>A</sub>	11.43 <sub>A</sub>	3.34 <sub>B</sub>	534.03 <sub>A</sub>	0.38 <sub>B</sub>
CV (%)	13.97	10.86	9.08	7.89	9.08	5.28
SE	1.0045	6.2469	0.1871	0.0610	8.7426	0.0044

Different letters indicate significant difference at the 1% probability level

### 5.3.6.3 Effects of ComCat treatments

The application of ComCat at different growth stages appeared to have no statistically significant effect on both vegetative growth (Fig. 5.5 A & B) and yield components (Table 5.15). The analysis of variance also confirmed the lack of significant differences among the different ComCat treatments in terms of all vegetative growth and yield parameters (Table 5.15).



**Figure 5.5:** Individual effects of ComCat treatments on vegetative growth parameters A) plant height and B) mass of air dry above ground parts as measured at harvest (values pooled for both water treatment and cultivars). Similar letters indicate non-significant differences among the ComCat levels at the 5 % probability level.

**Table 5.15:** Individual effects of ComCat treatments on yield and yield components of two Ethiopian wheat cultivars

ComCat levels	Number of kernels per spike	Total number of kernels per three plants	Fresh mass of air-dry kernels per three plants (g)	Hundred kernel mass (g)	Grain yield per unit area ( $\text{g m}^{-2}$ )	Harvest index
CC-0	35.69 <sup>A</sup>	294.33 <sup>A</sup>	10.44 <sup>A</sup>	3.69 <sup>A</sup>	487.95 <sup>A</sup>	0.41 <sup>A</sup>
CC-13	35.54 <sup>A</sup>	270.17 <sup>A</sup>	9.72 <sup>A</sup>	3.92 <sup>A</sup>	454.36 <sup>A</sup>	0.40 <sup>A</sup>
CC-19	34.78 <sup>A</sup>	280.33 <sup>A</sup>	10.15 <sup>A</sup>	3.79 <sup>A</sup>	474.06 <sup>A</sup>	0.40 <sup>A</sup>
CC-13 & 19	34.90 <sup>A</sup>	282.17 <sup>A</sup>	10.07 <sup>A</sup>	3.77 <sup>A</sup>	470.42 <sup>A</sup>	0.41 <sup>A</sup>
CV (%)	13.97	10.86	9.08	7.89	9.08	5.28
SE	1.4206	8.8345	0.2646	0.0863	12.3639	0.0062

Similar letters indicate non-significant differences at the 5% probability level.

#### 5.4 Discussion

In cereal crops, such as wheat, drought stress at certain stages of growth is more injurious than at other stages. In such crops, the critical period is usually just before reproductive organ formation or directly after pollination and fertilization and these stages are fundamentally important in determining the final grain yield (Gusta and Chen, 1987). This study attempted to investigate the influences of cultivar differences as well as post-anthesis water stress and ComCat treatments on vegetative growth and yield components in two Ethiopian wheat cultivars.

Direct interpretation of the acquired data indicated that ComCat treatment of HAR-2508 at all different growth stages tended to inhibit vegetative growth in terms of height in control plants under NWS conditions. This is consistent with claims made by the manufacturers (Agrorum, Germany -personal communication) namely that ComCat enhances root development in wheat but not the growth of above ground vegetative parts. Moreover, ComCat treatment of this cultivar at all growth stages tended to circumvent the growth inhibiting effect caused by the water stress treatment. This is also consistent with claims made by the manufacturers, namely that ComCat is a plant strengthening agent enhancing the natural resistance of wheat to stress conditions (personal communication). Exactly the same ComCat effect was observed in the tall cultivar, ET-13.

The above effect of ComCat was confirmed regarding the tendency to also reduce the second vegetative growth component, namely the air-dry mass of above ground parts, but only in HAR-2508 under NWS conditions. However, under WS conditions ComCat application tended to minimize the reduction in dry mass in this cultivar. Similarly, in the cultivar ET-13 under NWS conditions, dry mass of above ground parts remained more or less the same whether ComCat was applied or not. However, under WS conditions ComCat application, particularly the CC-19 treatment, tended to slightly increase the dry masses of above ground parts in ET-13 compared to the other treatments, including the untreated controls. The reduction in above ground part dry mass under NWS conditions could probably be attributed to an inhibiting effect of

ComCat on vegetative growth. However, the tendency of ComCat to minimize this reduction under WS conditions could probably not be explained along these lines but rather in terms of the possible ComCat effect on metabolism and substrate levels. The latter will be addressed in chapter 6.

The direct interpretation of the raw data for yield components indicated that the two cultivars showed distinctive tendencies in their responses to the ComCat and water treatments. In terms of the average number of kernels per spike as well as total number per plant, CC-13 and CC-19 ComCat treatments tended to enhance the average number of kernels in both HAR-2508 and ET-13 plants under NWS conditions. The early ComCat treatment (CC-13) also improved the hundred kernel mass in ET-13 under NWS, and in HAR-2508 under WS conditions. However, regarding the most important yield component i.e. grain yield per unit area, ComCat application statistically had no significant effect.

The positive effect of the ComCat application, more particularly of the earliest application (CC-13) on some of the yield components, appeared to be in agreement with the claims made by the manufacturers (personal communication). However, the inability of the ComCat treatment to increase the grain yield per unit area under glass house conditions was contrary to the field data obtained over three seasons (Pretorius J.C., 2000; personal communication; unpublished results) where the yield of a single South African field-grown wheat cultivar (SST825) was significantly improved through the foliar application of ComCat at growth stage 13. The lack of a positive influence of ComCat on yield in this study might have been due to the fact that the two cultivars were grown in relatively small pots. Under this condition the plants either faced a restriction in root development, compared to the case under the field condition or the improvement in root development had no beneficial effect due to the restriction placed on the plants by the pots in terms of extra water and nutrients.

Despite some of the positive effects of ComCat application, the analysis of variance revealed that all the observed differences in each of the cultivars under both conditions of water as well as ComCat treatments were not significant at the 5% probability level.

On the other hand, statistically significant interaction effects were observed in some of the combinations of any two of the three main factors. Cultivar X ComCat treatment interaction, albeit a slight one ( $P < 0.1$ ), was significant only in the total number of kernels per three plants grown in a pot. In this parameter, the highest number of kernels was produced in the ET-13 X CC-13 interaction, (i.e. the early application of ComCat), though it was not as such significantly different from the other interactions that involved the same cultivar and the remaining ComCat levels. Generally, more kernels were produced by the ET-13 X ComCat interaction than that by the HAR-2508 X ComCat interaction. The actual mechanism as to how this interaction influenced the formation of kernels appears to need further elaborate studies. However, the latter seems to coincide with the observations made by the manufacturers of ComCat in terms of its beneficial effect on flower bud formation (Agraforum, Germany; personal communication).

According to the statistical analyses procedure, it was the water X cultivar interaction that showed significant effects. Water deficit stress significantly reduced the most important yield parameter, namely grain yield per unit area in both cultivars. It was reported in the literature that post-anthesis water deficit stress resulted in severe yield reductions in cereals (Clark and Durley, 1981). The authors reported that in crops where the economic yield is the seed, moisture stress during any stage of reproductive development, including seed filling, has a direct influence on the final yield, depending on the severity and duration of the stress. Kobata *et al.* (1992) as well as Turner (1997) maintained that the principal cause for yield reductions due to post anthesis water stress is the fact that most of the photosynthate translocated during grain filling in cereals arises from photosynthesis in the spike, leaves and possibly even the stems.

According to Clark and Durley (1981) as well as Clarke *et al.* (1981), reductions in the supply of assimilate to the grain under drought conditions could be the result of either a reduction in the photosynthetic rate or a reduction in the photosynthesizing area or from reduced translocation of the photosynthate. This study revealed a positive and strong correlation ( $r = 0.95^{**}$ ) between total fresh mass of air-dry above ground parts and grain yield implicating any one or all three of the latter factors. In both cultivars

water stress reduced the dry mass of above ground parts but this reduction was more severe in ET-13 (-26%) than in HAR-2508 (-15%). The latter could probably be attributed to genetic differences between the cultivars. HAR-2508 is a relatively early maturing cultivar compared to ET-13, resulting in a shorter time of exposure to post anthesis water stress and probably less damage.

Post anthesis water deficit stress results in yield reduction also by probably affecting the size and weight of the kernels rather than by affecting the number of kernels produced (Shanahan *et al.*, 1984). This is probably because of the fact that, in the wheat plant, the final number of florets has already been established before anthesis (Simmons, 1987). In this study, however, the analysis of variance revealed a slightly significant reduction in the number of kernels produced ( $P < 0.1$ ) by ET-13 plants due to the stress condition, but this was not the case in the HAR-2508 plants. In addition to the cultivar X water treatment interaction effect, a statistical analysis of the individual effect of the water treatment also showed water stress significantly ( $P < 0.01$ ) reduced the number of kernels that were produced. The latter uncommon reduction in the number of kernels due to post anthesis water stress could be attributed to several factors. In this study, anthesis was determined when protrusions of anthers were observed in 50% of plants, after which the water stress treatment was applied. When the water stress was applied, it could be likely that development of some of the spikes of ET-13 plants, where protrusion of the anthers was delayed, could have been affected. This might have led to a situation where some of the florets were not fully developed and were therefore not yet fertilized when the stress began, due to the slower growth rate of ET-13 plants. The cultivar HAR-2508, owing to its inherent faster growth rate, appeared to have largely completed the fertilization process before the stress treatment commenced.

Moreover, Evans *et al.* (1975) reported that, due to the influences of several factors, all florets reaching anthesis might not yet have set grains. The authors mentioned factors such as the short life span (few hours) of wheat pollen, which sometimes make synchronization with pollination difficult and the fact that pollination is also of short duration (1 to 1.5 hours). The latter might be further complicated by the very short

duration in which the florets remain open (8 to 30 minutes; Simmons, 1987). The ET-13 plants might have been more exposed to such factors than HAR-2508 due to their longer life cycle.

The hundred kernel mass was significantly higher in plants of the cultivar HAR-2508 than that of ET-13, despite the higher yield per unit area of the latter. The mass of kernels appeared to be associated with the number of kernels produced as the correlation analysis revealed a strong but negative correlation between the number of kernels and the hundred kernel mass, indicating that the higher the number of kernels the lower the mass of individual grains. This is in agreement with the reports of Fisher *et al.* (1977) and Shanahan *et al.* (1984) who found kernel weight falling linearly with an increase in grain number. This negative correlation between the two parameters might be due to the competition for assimilate among the grains. It is obvious that the reduction in the photosynthesizing area (due to premature senescence of leaves) reduces the overall supply of assimilates (Clarke *et al.*, 1981), leading to increased competition among the developing grains. Similar results were also reported by Pannozo and Eagles (1998) who observed a reduction in the rate of grain filling in wheat under water stress.

Moreover, the statistical analysis of the cultivar X water treatment interaction revealed that, of all the parameters measured, it was in plant height and harvest index that no significant interaction effects were observed. The lack of significant difference in height is very likely to be due to the termination of vegetative growth after anthesis (Clark and Durley, 1981; Simmons 1987). The 8% and 5% reduction in harvest index in ET-13 and HAR-2508 respectively was not statistically significant indicating that the rate of translocation of assimilates to the developing grains was more or less similar under both levels of water treatment and in both cultivars. It appears, therefore, that each of the cultivars didn't show any special capacity to speed up translocation of assimilates to the developing grains under water stress.

Statistical analysis of the individual effects of the main factors also provided important information. When mean values of parameters were pooled for cultivars and ComCat

levels, significant differences were observed in each of the parameters, except in plant height, between NWS and WS treatments. Water stress consistently reduced the measured values of all these parameters. Statistical analysis of the individual effects of cultivar differences also revealed that ET-13 was significantly superior ( $P < 0.001$ ) to HAR-2508 in most of the growth and yield parameters including height, total mass of air-dry above ground parts, number of kernels per spike, total number of kernels, fresh mass of air-dry kernels and grain yield per unit area. On the other hand, HAR-2508 was superior to ET-13 ( $P < 0.001$ ) in hundred kernel mass and harvest index. These observations indicate the significant differences in the genetic make up of these two cultivars. Similar genetic differences among other wheat cultivars were also reported by Clarke *et al.* (1981) as well as Belay *et al.* (1993) in terms of both vegetative growth and yield components.

Of the three main factors, it was the ComCat treatment that showed no significant individual effect in terms of all the measured parameters under glass house conditions. However, as mentioned earlier, in field experiments (Pretorius J.C. - personal communication; unpublished results) during the 1998 and 1999 seasons, significant yield improvements were observed in both wheat and maize cultivars treated with ComCat in a similar dose as the one used in this study. The most significant yield improvement was obtained in 1998, a season which was characterized as extremely dry and with abnormally high temperatures, compared to that of 1999. Based on the two years work it was recommended to apply ComCat on wheat as a foliar spray at the three-leaf stage (growth stage 13) at a dose as used in this study.

In summary, both wheat cultivars were negatively affected by the post anthesis drought stress treatment in terms of both vegetative growth and yield parameters. However, treatment with ComCat tended to minimize or even reduce the devastating effect of the water deficit condition especially in terms of the yield parameters, in both wheat cultivars. In an attempt to further understand how ComCat might have interfered in minimizing the deleterious effects of the water deficit stress, metabolic processes, specifically those leading to changes in protein and starch content were considered in Chapter 6.

## Chapter 6

### **Influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on protein and starch contents in seed of two Ethiopian wheat cultivars**

#### **6.1 Introduction**

Common wheat (*Triticum aestivum*) is grown across a wide range of environments around the world and shows the broadest adaptation of all the cereal crop species (Briggle and Curtis, 1987). This broad adaptation has contributed to wheat being among the few most important crops that feed the ever-increasing human population. Although wheat is relatively low in protein content compared to legume crops (Payne, 1987), it still remains one of the major sources of protein to humans and animals. The protein content of wheat, which is between 7 to 15 % of grain dry (Shewry and Miffin, 1985), is influenced primarily by environmental conditions during growth as well as by the genotype and the interaction of the genotype with the growth environment (Donovan *et al.*, 1977). Similarly, starch metabolism is also affected by environmental and genetic factors. According to Pannozzo and Eagles (1998), starch composition is one of the determinants of grain quality, which depends on cultivar and the environment in which the crop is grown.

This chapter deals with the influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on the levels of protein and starch in seed of two Ethiopian wheat cultivars. By applying ComCat at different growth stages, an attempt was made to alleviate the possible negative effects of the water stress on seed quality.

## **6.2 Materials and Methods**

Refer to chapter 3; sections 3.1 and 3.2.

## **6.3 Results**

### **6.3.1 Influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on the protein content in seed of two Ethiopian wheat cultivars**

#### **6.3.1.1 Water soluble protein content (WSP)**

ComCat application at different growth stages did not show an appreciable effect on the WSP content ( $\text{mg g}^{-1}$  fresh mass of seed, FW) in the cultivar HAR-2508 under the NWS condition, while under the WS condition, particularly the early application of ComCat (CC-13), resulted in a considerable reduction (32%) compared to that of the untreated control plants (Table 6.1).

On a  $\text{mg kernel}^{-1}$  basis, the WSP content in a single seed of HAR-2508 grown under NWS conditions appeared to have been slightly increased by ComCat treatment and more particularly by the double application (CC-13 & 19) (Table 6.2). However, the reverse trend occurred under WS conditions, where ComCat treatment tended to either decrease the WSP content (CC-13 and CC-13 & 19) or not affect it at all (CC-19). The latter was consistent with the trend observed on a  $\text{mg g}^{-1}$  FW basis (Table 6.1).

**Table 6.1:** Influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on water soluble protein content in seeds of two Ethiopian wheat cultivars, as expressed on a  $\text{mg g}^{-1}$  FW basis.

ComCat treatments	WSP content in seeds ( $\text{mg g}^{-1}$ FW)			
	HAR-2508		ET-13	
	NWS	WS	NWS	WS
CC-0	$33.61 \pm 12.34$ A	$43.95 \pm 10.99$ A	$33.54 \pm 4.51$ A	$45.20 \pm 11.37$ A
CC-13	$32.15 \pm 5.85$ A	$30.00 \pm 7.63$ A	$29.77 \pm 8.93$ A	$36.36 \pm 11.54$ A
CC-19	$32.83 \pm 4.85$ A	$41.88 \pm 9.51$ A	$32.09 \pm 7.56$ A	$32.06 \pm 7.79$ A
CC-13&19	$34.55 \pm 4.76$ A	$41.05 \pm 17.83$ A	$28.15 \pm 8.08$ A	$36.00 \pm 13.33$ A
CV (%)	19.02			
SE	3.8652			

Similar letters in front of mean values indicate non-significant differences among the means at the 5 % probability level.  $\pm$  = Standard deviation for three replications.

In the cultivar ET-13 grown under NWS conditions, the WSP content ( $\text{mg g}^{-1}$  FW) was reduced by all of the ComCat applications, but more markedly by the CC-13 & 19 (-16%) and CC-13 (-11%) treatments compared to that of the untreated control plants (Table 6.1). The same trend of reduction of WSP content under the influence of ComCat was observed in this cultivar under WS conditions and this was more pronounced than in HAR-2508. Reductions in WSP content of -20%, -29% and -20% were observed in the CC-13, CC-19 and CC-13 & 19 ComCat treated plants, respectively, compared to that of the untreated controls. Similarly, on a  $\text{mg seed}^{-1}$  basis (Table 6.2) the same trend was observed in ET-13 under both NWS and WS conditions. However, in ET-13 these reductions in the WSP content were more

pronounced under WS conditions where the CC-13, CC-19 and CC-13 & 19 ComCat treatments caused reductions of -26%, -28% and -26%, respectively as compared to that of the untreated controls.

**Table 6.2:** Influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on water soluble protein content in seeds of two Ethiopian wheat cultivars, as expressed on mg seed<sup>-1</sup> basis.

ComCat treatments	WSP content (mg seed <sup>-1</sup> )			
	HAR-2508		ET-13	
	NWS	WS	NWS	WS
CC-0	1.36 ± 0.51 A	1.41 ± 0.22 A	1.07 ± 0.11 A	1.16 ± 0.29 A
CC-13	1.38 ± 0.27 A	1.15 ± 0.27 A	1.00 ± 0.26 A	0.86 ± 0.22 A
CC-19	1.21 ± 0.31 A	1.42 ± 0.09 A	1.01 ± 0.27 A	0.84 ± 0.15 A
CC-13&19	1.44 ± 0.14 A	1.34 ± 0.44 A	0.93 ± 0.27 A	0.86 ± 0.20 A
CV (%)	18.07			
SE	0.1202			

Similar letters in front of mean values indicate non-significant differences among the means at the 5 % probability level. ± = Standard deviation for three replications.

However, according to the analysis of variance, despite some of the substantial differences and clear patterns, all the observed variations in WSP content on both mg g<sup>-1</sup> FW and mg seed<sup>-1</sup> basis were non-significant at the 5% probability level for both cultivars under both conditions of water treatment.

The water stress treatment *per se*, on the other hand, caused a general increase in the WSP content of > 30% in plants not treated with ComCat and in both cultivars

(Table 6.1). It also increased the WSP content in the other ComCat treated plants in both

cultivars. These increases in the cultivar HAR-2508 were +28% and +19 % in CC-19 and CC-13 & 19 ComCat treated plants, respectively. However, no appreciable differences occurred due to the water stress in CC-13 treated plants of the same cultivar. From this it seemed that especially the early ComCat treatment (CC-13) either reduced the synthesis of water soluble proteins or the breakdown of non-water soluble proteins. The increases in WSP content in the cultivar ET-13 were +35%, +22% and +28% in the untreated controls, CC-13 and CC-13 & 19 treated plants, respectively (Table 6.1). On a  $\text{mg seed}^{-1}$  basis (Table 6.2), the reducing effect of the water stress treatment on the WSP content followed the same trend in HAR-2508. In ET-13 and on a  $\text{mg seed}^{-1}$  basis, the WSP content showed a general reduction in seeds of all ComCat treated plants (Table 6.2). However, none of the observed differences were statistically significant at the 5% probability level.

#### 6.3.1.2 Total protein content (TPC)

In the cultivar HAR-2508 grown under NWS conditions, all ComCat applications increased the total seed protein content, measured based on total Nitrogen, markedly compared to that of untreated controls (Table 6.3). A similar trend was observed in the seeds of ComCat treated HAR-2508 plants under WS conditions but the increases were less marked (Table 6.3). Although the TPC in seeds was >20% in some instances (CC-13 under NWS and CC-19 under WS conditions), this was not statistically significant.

**Table 6.3:** Influences of cultivar difference, post-anthesis water stress as well as ComCat treatments on total protein content in seeds of two Ethiopian wheat cultivars, as expressed on a mg g<sup>-1</sup> FW basis.

ComCat treatments	Total protein content in seeds (mg g <sup>-1</sup> FW)			
	HAR-2508		ET-13	
	NWS	WS	NWS	WS
CC-0	131.25 ± 4.30 A	152.99 ± 18.27A	104.65 ± 8.29 A	142.98 ± 28.25 A
CC-13	161.74 ± 21.80 A	168.19 ± 22.89A	104.45 ± 13.42 A	153.27 ± 30.61A
CC-19	141.74 ± 16.10 A	190.76 ± 15.51A	102.22 ± 12.93 A	141.25 ± 19.07 A
CC-13&19	148.89 ± 16.99 A	160.97 ± 16.37 A	103.12 ± 7.45 A	146.81 ± 24.07A
CV (%)			9.78	
SE			7.9616	

Similar letters in front of mean values indicate non-significant differences among the means at the 5 % probability level. ± = Standard deviation for three replications.

Exactly the same tendency of ComCat treatments to increase the total protein content in HAR-2508 seeds was observed on a mg seed<sup>-1</sup> basis (Table 6.4) under both NWS and WS conditions.

On the other hand, in the cultivar ET-13, the total protein content remained largely unaffected by the ComCat treatments under both conditions of water treatment and as expressed both on a mg g<sup>-1</sup> FW (Table 6.3) and mg seed<sup>-1</sup> (Table 6.4) basis.

**Table 6.4:** Influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on total protein content in seeds of two Ethiopian wheat cultivars, as expressed on a mg seed<sup>-1</sup> of seeds basis.

ComCat treatments	Total protein content (mg seed <sup>-1</sup> )			
	HAR-2508		ET-13	
	NWS	WS	NWS	WS
CC-0	6.11 ± 0.44 A	5.57 ± 0.49 A	3.71 ± 0.25 A	4.02 ± 0.79A
CC-13	7.65 ± 1.36 A	7.25 ± 0.91A	3.94 ± 0.71A	4.37 ± 0.78A
CC-19	6.24 ± 1.52 A	7.38 ± 1.41A	3.79 ± 0.39A	4.27 ± 0.91A
CC-13&19	6.89 ± 0.29 A	6.09 ± 0.60A	3.91 ± 0.45A	4.16 ± 0.88A
CV (%)	14.08			
SE	0.4336			

Similar letters in front of mean values indicate non-significant differences among the means at the 5 % probability level. ± = Standard deviation for three replications.

As was the case with water soluble protein (WSP), the water stress treatment *per se* resulted in considerable increases in the total protein content (TPC) of seeds of both cultivars, whether ComCat was applied or not and when TPC was expressed on a mg g<sup>-1</sup> FW basis (Table 6.3).

These increases in total protein content were +17% in seeds of untreated controls and +4% in CC-13, +35% in CC-19, and +8% in CC-13 & 19 ComCat treated HAR-2508 plants. In seeds of the cultivar ET-13 the TPC increases were more marked namely +37% in untreated controls and +47% in CC-13, +38% in CC-19, and +42% in CC-13 & 19 ComCat treated plants (Table 6.3). However, on a mg seed<sup>-1</sup> basis, (Table 6.4) the observed increases in TPC was less pronounced in seeds of both cultivars under WS conditions. Despite of the fact that increases in TPC of seeds

were as high as 42%, the analysis of variance revealed no significant differences at the 5% probability level. In light of the difficulty to contemplate the latter inability of the statistical procedure to discriminate between rather substantial numerical differences in TCP in seeds, statistical analyses were performed from different perspectives in order to verify this apparent discrepancy.

### **6.3.2 Analysis of the correlation between protein content in seeds and some yield parameters**

In order to identify the possible relationships between seed protein content parameters among themselves, as well as between seed protein content and some yield parameters, a correlation analysis was performed (data pooled over three replications for each of the three main factors in each parameter). This correlation analysis indicated that protein content was closely related to other yield parameters.

As shown in Table 6.5, the average water soluble protein (WSP) content in a single seed was strongly and positively correlated ( $P < 0.01$ ) with the WSP and total protein content in a gram of seed as well as with the total protein content in a single seed and with the average single kernel weight. It, however, negatively but strongly correlated ( $P < 0.01$ ) with grain yield per unit area as well as with number of kernels per three plants (grown in a pot). On the other hand, WSP content in a gram of seed was strongly and positively correlated ( $P < 0.01$ ) with total protein content in a gram of seed. It was also strongly but negatively correlated with grain yield per unit area as well as with average single kernel weight. Similarly, total protein content in a gram of seed strongly but negatively correlated ( $P < 0.01$ ) with grain yield per unit area as well as with number of kernels per three plants. Its correlation with average single kernel weight was not significant at the 5% probability level.

**Table 6.5:** Correlation of grain protein content, as expressed on both  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis, with some grain yield parameters.

	WSP ( $\text{mg g}^{-1}$ FW)	WSP ( $\text{mg seed}^{-1}$ )	Total protein ( $\text{mg g}^{-1}$ FW)	Total protein ( $\text{mg seed}^{-1}$ )
WSP ( $\text{mg seed}^{-1}$ )	0.69**			
Total protein ( $\text{mg g}^{-1}$ FW)	0.51**	0.49**		
Total protein ( $\text{mg seed}^{-1}$ )	0.055 <sup>ns</sup>	0.59**	0.70**	
Grain yield per unit area	-0.41**	-0.36**	-0.86**	-0.57**
Number of kernels per three plants in one pot	-0.13 <sup>ns</sup>	-0.57**	-0.70**	-0.90**
Average single kernel weight	-0.33*	0.41**	0.06 <sup>ns</sup>	0.74**

\*, \*\* = Correlation significant at the 5% and 1% probability levels respectively.  
ns = correlation non-significant at the 5% probability level.

### 6.3.3 Statistical analysis of the effect of cultivar X ComCat treatment interaction on the protein content in seeds of two Ethiopian wheat cultivars

#### Water soluble protein (WSP) content

**Table 6.6:** Statistical analysis of the effect of cultivar X ComCat treatment interaction on water soluble protein content in seeds of two Ethiopian wheat cultivars, as expressed on both mg g<sup>-1</sup> FW and mg seed<sup>-1</sup> basis.

ComCat treatments	WSP content (mg g <sup>-1</sup> FW)		WSP content (mg seed <sup>-1</sup> )	
	HAR-2508	ET-13	HAR-2508	ET-13
CC-0	38.78 A	39.37 A	1.38 A	1.11 A
CC-13	31.08 A	33.06 A	1.27 A	0.93 A
CC-19	37.36 A	32.08 A	1.31 A	0.92 A
CC-13 & 19	37.80 A	32.07 A	1.39 A	0.90 A
CV (%)	19.02		18.07	
SE	2.7331		0.085	

Similar letters in front of mean values indicate non-significant differences among the means at the 5 % probability level.

From this interaction (data pooled for both water treatments), the earliest application of ComCat (CC-13) tended to reduce the WSP content in seeds of both cultivars, while no appreciable differences were observed for the CC-19 and CC-13 & 19 ComCat treatments on a mg g<sup>-1</sup> FW basis, as compared to that of the untreated

control plants (Table 6.6). On a  $\text{mg seed}^{-1}$  basis, however, the tendency of ComCat to reduce the WSP content in seeds was the same for seeds of ET-13 but less pronounced in seeds of HAR-2508. According to the analysis of variance these differences were not statistically significant at the 5% probability level for both cultivars and with regard to both ways in which the WSP content were expressed.

### Total protein content

**Table 6.7:** Statistical analysis of the effect of cultivar X ComCat treatment interaction on total protein content in seeds of two Ethiopian wheat cultivars, as expressed on both a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis.

ComCat treatments	Total protein content ( $\text{mg g}^{-1}$ FW)		Total protein content ( $\text{mg seed}^{-1}$ )	
	HAR-2508	ET-13	HAR-2508	ET-13
CC-0	142.12 <sup>A</sup>	123.83 <sup>A</sup>	5.84 <sup>A</sup>	3.86 <sup>A</sup>
CC-13	164.97 <sup>A</sup>	128.86 <sup>A</sup>	7.45 <sup>A</sup>	4.16 <sup>A</sup>
CC-19	166.25 <sup>A</sup>	121.74 <sup>A</sup>	6.81 <sup>A</sup>	4.03 <sup>A</sup>
CC-13 & 19	154.93 <sup>A</sup>	124.97 <sup>A</sup>	6.49 <sup>A</sup>	4.04 <sup>A</sup>
CV (%)	9.78		14.08	
SE	5.6297		0.3066	

Similar letters in front of mean values indicate non-significant differences among the means at the 5 % probability level.

The application of ComCat appeared to have an enhancing effect on the total protein content in seeds of HAR-2508 (data pooled over both levels of water treatment) as expressed on both a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis (Table 6.7). In seeds of ET-13, however, ComCat treatment had no appreciable effect on the total protein content expressed on a  $\text{mg g}^{-1}$  FW basis, but on a  $\text{mg seed}^{-1}$  basis the same enhancing

effect was observed in seeds of the same cultivar, as was the case for seeds of HAR-2508 (Table 6.7). The analysis of variance, however, revealed no significant differences in total protein content at the 5% probability level in both cultivars, and for both ways in which the total protein content were expressed.

#### 6.3.4 Statistical analysis of the effect of water X ComCat treatment interaction on the protein content in seeds of two Ethiopian wheat cultivars

##### Water soluble protein content

**Table 6.8:** Statistical analysis of the effect of water X ComCat treatment interaction on water soluble protein content in seeds of two Ethiopian wheat cultivars, as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis.

ComCat treatments	WSP content ( $\text{mg g}^{-1}$ FW)		WSP ( $\text{mg seed}^{-1}$ )	
	NWS	WS	NWS	WS
CC-0	33.57 A	44.58 A	1.22 A	1.28 A
CC-13	30.96 A	33.18 A	1.19 A	1.01 A
CC-19	32.46 A	36.97 A	1.11 A	1.13 A
CC-13 & 19	31.35 A	38.52 A	1.18 A	1.10 A
CV (%)	19.02		18.07	
SE	2.7331		0.085	

Similar letters in front of mean values indicate non-significant differences at the 5 % probability level.

Statistically, the interaction between water and ComCat treatments (data pooled for cultivars) showed no significant effect on WSP content in seeds, as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis (Table 6.8). However, the water stress treatment caused increases in WSP content as expressed on a  $\text{mg g}^{-1}$  FW basis whether ComCat was applied or not, but not in terms of  $\text{mg seed}^{-1}$  basis. In the former, increases of +33%, +7%, +14% and +23% were observed in seeds of untreated controls and CC-13, CC-19, and CC-13 & 19 ComCat treated plants, respectively.

### Total protein content

**Table 6.9:** Statistical analysis of the effect of water X ComCat treatment interaction on total protein content in seeds of two Ethiopian wheat cultivars, as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis.

ComCat treatments	Total protein content ( $\text{mg g}^{-1}$ FW)		Total protein content ( $\text{mg seed}^{-1}$ )	
	NWS	WS	NWS	WS
CC-0	117.95 A	147.99 A	4.91 A	4.79 A
CC-13	133.09 A	160.73 A	5.79 A	5.81 A
CC-19	121.98 A	166.01 A	5.02 A	5.82 A
CC-13 & 19	126.01 A	153.89 A	5.40 A	5.13 A
CV (%)	9.78		14.08	
SE	5.6297		0.3066	

Similar letters in front of mean values indicate non-significant differences among the means at the 5 % probability level.

In contrast to the WSP content, total protein content was slightly enhanced by the ComCat treatments under both conditions of water treatment (data pooled for both cultivars) as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis.

The water stress *per se* also increased the TPC ( $\text{mg g}^{-1}$  FW), but there was no consistent pattern as expressed on a  $\text{mg seed}^{-1}$  basis (Table 6.9). In the former, increases of +25%, +21%, +36% and +22% in seeds of untreated controls and CC-13, CC-19 and CC-13 & 19 ComCat treated plants, respectively, were observed as a result of the water stress. Despite these tendencies, the observed differences in TPC among the ComCat treatments as well as in non-water stress and stressed plants were statistically non-significant at the 5% probability level.

### 6.3.5 Statistical analysis of the effect of cultivar X water treatment interaction on the protein content in seeds of two Ethiopian wheat cultivars

#### Water soluble protein content

**Table 6.10:** Statistical analysis of the effect of water X cultivar interaction on water soluble protein content in seeds of two Ethiopian wheat cultivars, as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis.

Cultivars	Water soluble protein content ( $\text{mg g}^{-1}$ FW)		Water soluble protein content ( $\text{mg seed}^{-1}$ )	
	NWS	WS	NWS	WS
HAR-2508	33.29 <sub>AB</sub>	39.22 <sub>A</sub>	1.35 <sub>A</sub>	1.33 <sub>A</sub>
ET-13	30.89 <sub>B</sub>	37.40 <sub>AB</sub>	1.00 <sub>B</sub>	0.93 <sub>B</sub>
CV (%)	19.02		18.07	
SE	1.9326		0.0601	

Different letters indicate significant differences, while similar letters indicate non-significant differences at the 5% probability level.

Under both conditions of water treatment, seeds of the cultivar HAR-2508 were superior in WSP content to that of ET-13, as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis (Table 6.10). On the other hand, the water stress treatment increased the WSP content in both cultivars, as expressed on a  $\text{mg g}^{-1}$  FW basis, but on  $\text{mg seed}^{-1}$  basis a slight and non-significant reduction was observed in both cultivars (Table 6.10)

### Total protein content

**Table 6.11:** Statistical analysis of the effect of water X cultivar interaction on total protein content in seeds of two Ethiopian wheat cultivars, as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis.

Cultivars	Total protein content ( $\text{mg g}^{-1}$ FW)		Total protein content ( $\text{mg seed}^{-1}$ )	
	NWS	WS	NWS	WS
HAR-2508	145.90 <sub>b</sub>	168.23 <sub>a</sub>	6.72 <sub>a</sub>	6.57 <sub>a</sub>
ET-13	103.61 <sub>c</sub>	146.08 <sub>b</sub>	3.84 <sub>b</sub>	4.21 <sub>b</sub>
CV (%)	9.78		14.08	
SE	3.9808		0.2168	

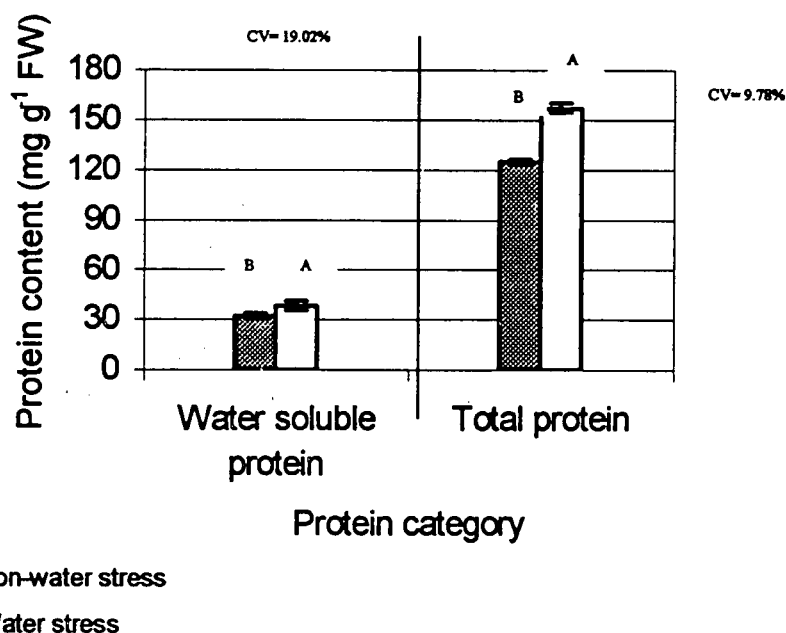
Different letters indicate significant differences, while similar letters indicate non-significant differences at the 5 % probability level.

Similar to the case observed in WSP content, the total protein content was also significantly higher ( $P < 0.05$ ) in seeds from the cultivar HAR-2508 compared to that of seeds of ET-13 plants under both conditions of water treatment, as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis (Table 6.11). On the other hand, total protein content, as expressed on a  $\text{mg g}^{-1}$  FW basis, but not on a  $\text{mg seed}^{-1}$  basis, was significantly ( $P < 0.05$ ) increased by the water stress treatment in both cultivars (Table 6.11). These increases were +15% and +41% in seeds of HAR-2508 and ET-13 plants respectively.

### 6.3.6 Statistical analysis of individual effects of cultivar difference, water as well as ComCat treatments on the protein content in seed of two Ethiopian wheat cultivars

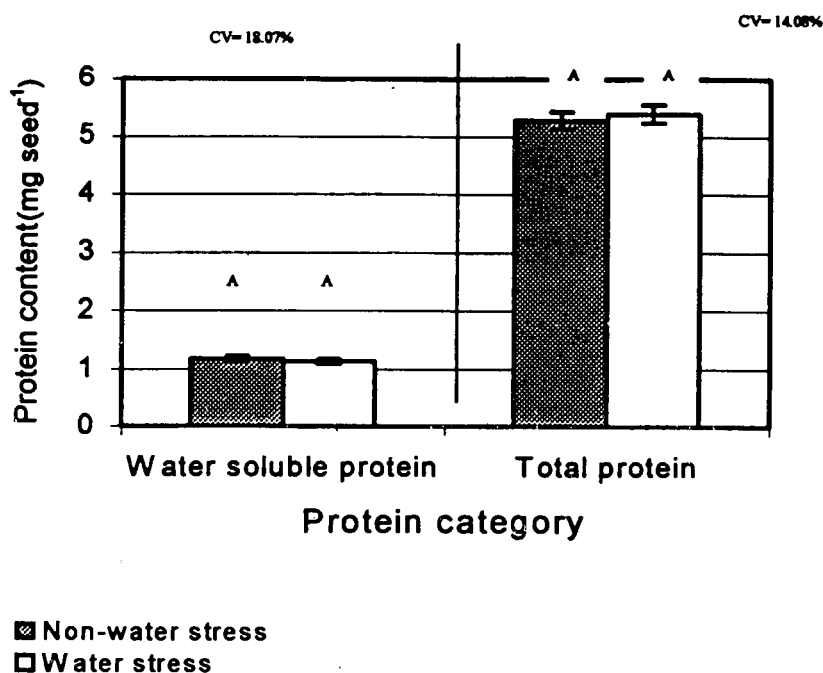
#### 6.3.6.1 Effect of water treatment (data pooled for cultivars and ComCat treatments)

The water stress treatment significantly increased ( $P < 0.05$ ) the water soluble and total protein contents as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis (Figure 6.1). The increases were +19% and 26% in WSP and TPC, respectively.



**Figure 6.1:** Individual effects of water treatment on water soluble and total protein content in seeds of two Ethiopian wheat cultivars, as expressed on a  $\text{mg g}^{-1}$  FW basis. (Different letters on top of the columns indicate significant difference at the 1% Probability level).

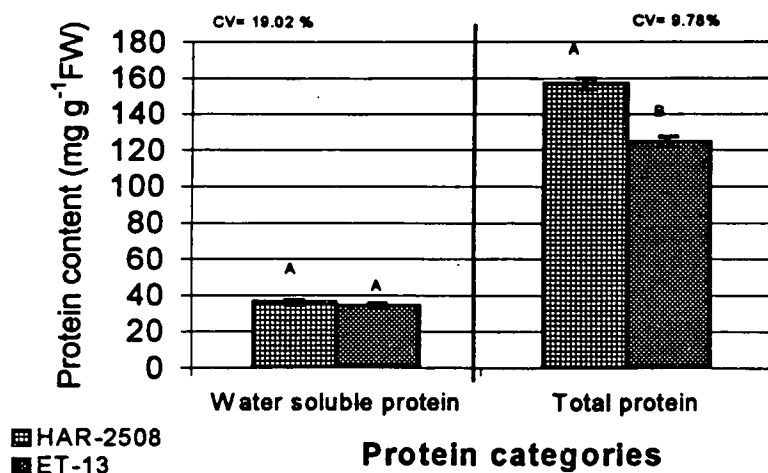
On the other hand, the WSP content, as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis did not significantly vary between seeds from water-stressed and non-stressed plants, at the 5% probability level (Figure 6.2).



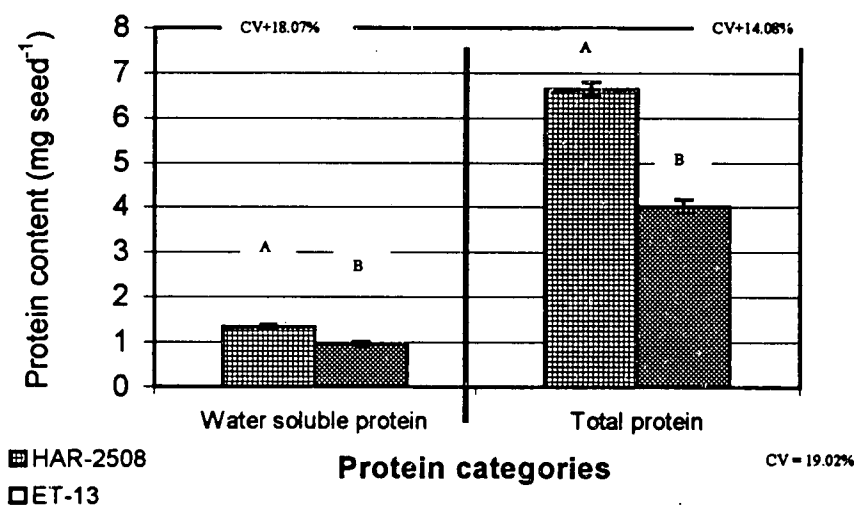
**Figure 6.2 :** Individual effects of water treatment on the average water soluble and total protein contents in seeds of two Ethiopian wheat cultivars, as expressed on a  $\text{mg seed}^{-1}$  basis. (Similar letters on top of the columns indicate non-significant difference at the 5 %Probability level.)

#### 6.3.6.2 Effect of cultivar differences (data pooled for water and ComCat treatments)

Except in the WSP content ( $\text{mg g}^{-1}$  FW), there were significant differences between the two cultivars. Seeds of HAR-2508 were superior to those of ET-13 in total protein content (+26%), as expressed on a  $\text{mg g}^{-1}$  FW basis (Figure 6.3), and WSP content (+39%) and TPC (+38%), as expressed on a  $\text{mg seed}^{-1}$  basis (Figure 6.4).



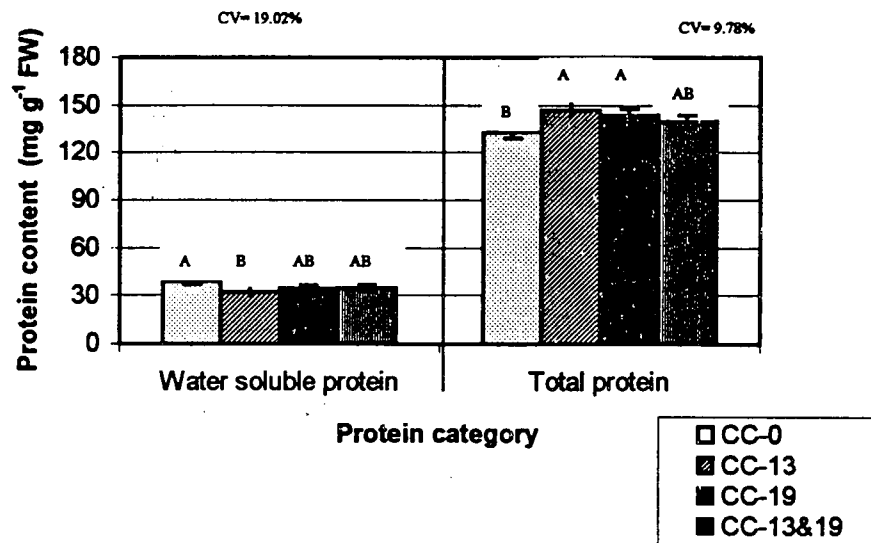
**Figure 6.3:** Effects of cultivar differences on water soluble and total protein content in seeds of two Ethiopian wheat cultivars, as expressed on a mg g<sup>-1</sup> FW basis. (Different letters indicate significant difference at the 1% probability level, while similar letters indicate non-significant difference at the 5% probability level).



**Figure 6.4:** Effects of cultivar differences on water soluble, and total protein content in seeds of two Ethiopian wheat cultivars as expressed on a mg seed<sup>-1</sup> basis. (Different letters on top of the columns indicate significant difference at the 1% probability level, while similar letters indicate otherwise).

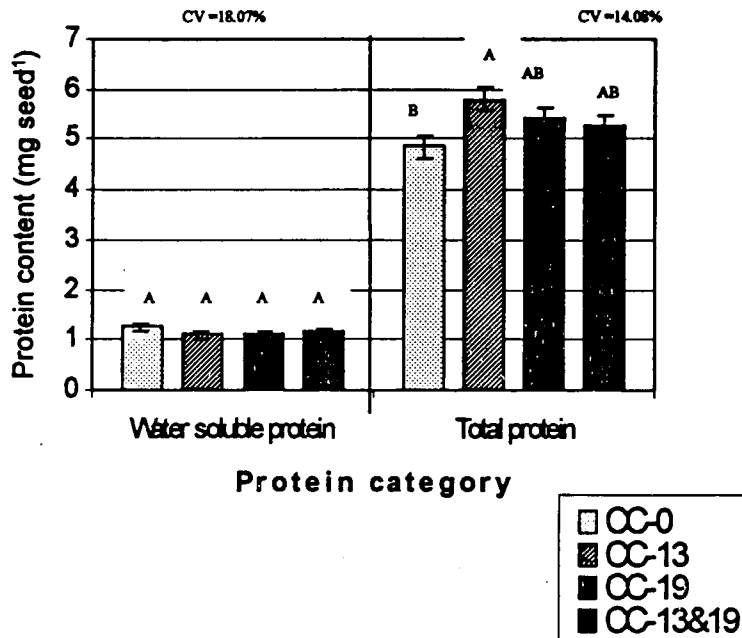
### 6.3.6.3 Effect of ComCat treatments (data pooled for cultivars and water treatments)

ComCat application, more particularly the early application (CC-13) showed a tendency to reduce the WSP content, while it significantly ( $P < 0.01$ ) increased the total protein content, as expressed on a  $\text{mg g}^{-1}$  FW basis (Figure 6.5).



**Figure 6.5:** Effects of ComCat treatments on water soluble and total protein content in seeds of two Ethiopian wheat cultivars, as expressed on a  $\text{mg g}^{-1}$  FW basis. (Different letters on top of the columns indicate significant difference at the 1% probability level, while similar letters indicate otherwise).

On the other hand, ComCat application at different growth stages made no significant difference in WSP content, while it made a definite difference in terms of total protein content, both expressed on a mg seed<sup>-1</sup> basis (Figure 6.6). In the latter, particularly the early application (CC-13) resulted in a significant increase ( $P < 0.05$ ) in total protein content compared to that in untreated control plants.



**Figure 6.6:** Effects of ComCat treatments on water soluble and total protein content in seeds of two Ethiopian wheat cultivars, as expressed on a mg seed<sup>-1</sup> basis. (Different letters on top of the columns, in total protein, indicate significant differences at the 5 % probability level, while similar letters, in WSP content indicate non-significant difference).

### 6.3.7 Influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on the starch content in seeds of two Ethiopian wheat cultivars

**Table 6.12:** Influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on starch content in seeds of two Ethiopian wheat cultivars, as expressed on a  $\text{g g}^{-1}$  FW basis.

ComCat treatments	Starch content ( $\text{g g}^{-1}$ FW)			
	HAR-2508		ET-13	
	Nws	WS	Nws	WS
CC-0	$0.60 \pm 0.04$ ABCDE	$0.54 \pm 0.04$ DE	$0.63 \pm 0.08$ ABCD	$0.64 \pm 0.04$ AB
CC-13	$0.60 \pm 0.03$ ABCDE	$0.52 \pm 0.04$ E	$0.62 \pm 0.02$ ABCDE	$0.54 \pm 0.03$ CDE
CC-19	$0.58 \pm 0.05$ BCDE	$0.61 \pm 0.07$ ABCDE	$0.69 \pm 0.03$ A	$0.58 \pm 0.03$ BCDE
CC-13&19	$0.66 \pm 0.02$ AB	$0.63 \pm 0.02$ ABCD	$0.64 \pm 0.03$ ABC	$0.53 \pm 0.03$ DE
CV (%)	6.22			
SE	0.0216			

Similar letters in front of mean values indicate non-significant differences at the 5 % probability level, while different letters indicate significant differences.  $\pm$  = Standard deviation for three replications.

ComCat application tended to slightly enhance starch content, as expressed both on a  $\text{g g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis under the NWS condition in both cultivars and only in HAR-2508 under the WS condition. The highest starch content under NWS conditions in the cultivar HAR-2508, both on a  $\text{g g}^{-1}$  FW (Table 6.12) and  $\text{mg seed}^{-1}$  basis (Table 6.13), occurred in seeds of CC-13 & 19 treated plants compared to that of the other ComCat treatments, including the untreated control plants. Under the water stress condition, however, CC-13 & 19 and CC-19 treated plants produced seeds with the highest starch contents as expressed on a  $\text{g g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis, respectively. On the other hand, in the cultivar ET-13, under non-water stress conditions, the CC-19 treatment produced seeds with the highest starch content ( $\text{g g}^{-1}$  FW) compared to that of the other ComCat treatments, including the untreated control plants (Table 6.12). Under the water stress condition, however, in contrast to what was observed in seeds of HAR-2508, ComCat application tended to slightly reduce the starch content as expressed both on a  $\text{g g}^{-1}$  FW (Table 6.12) and  $\text{mg seed}^{-1}$  basis (Table 6.13) in ET-13. The reductions, compared to seeds of the untreated control plants (as expressed on a  $\text{g g}^{-1}$  FW basis) were -16%, -9% and -17% in CC-13, CC-19 and CC-13 & 19 treated plants respectively, and except for CC-19 treated plants, these differences were significant ( $P < 0.05$ ). On the other hand, the differences observed in starch content, as expressed in  $\text{mg seed}^{-1}$  basis, were not statistically significant for both cultivars under both conditions of water treatment at the 5% probability level.

**Table 6.13:** Influences of cultivar differences, post-anthesis water stress as well as ComCat treatments on starch content, in seeds of two Ethiopian wheat cultivars, as expressed on a mg seed<sup>-1</sup> basis.

ComCat treatments	Starch content (mg seed <sup>-1</sup> )			
	HAR-2508		ET-13	
	Nws	WS	Nws	WS
CC-0	27.49 ± 2.88 A	23.14 ± 1.20 A	22.71 ± 1.75 A	19.36 ± 2.52 A
CC-13	29.18 ± 2.52 A	22.62 ± 3.75 A	24.87 ± 1.04 A	16.00 ± 0.81 A
CC-19	28.26 ± 4.38	27.23 ± 4.86 A	26.68 ± 2.38 A	19.36 ± 3.44 A
CC-13&19	33.10 ± 2.00 A	25.89 ± 1.80 A	26.07 ± 1.54 A	17.07 ± 0.74 A
CV (%)				10.36
SE				1.4543

Similar letters in front of mean values indicate non-significant difference at the 5 % probability level. ± = Standard deviation for three replications.

With regard to the effect of the water stress, a general reduction in starch content as expressed both on a g g<sup>-1</sup> FW (Table 6.12) and mg seed<sup>-1</sup> (Table 6.13) basis was observed in both cultivars. Furthermore, as can be seen in both Tables, reductions under the water stress was more severe in ET-13 than in HAR-2508. Particularly in the cultivar HAR-2508, the CC-19 treatment tended to even prevent the reduction in starch content almost completely.

### 6.3.8 Analysis of correlation between grain starch content and some yield parameters.

The correlation analysis (values pooled over three replications of all the combinations of the three main factors; Table 6.14) shed some light on the relationships between starch content and yield parameters. The analysis revealed that starch content ( $\text{g g}^{-1}$  FW) was strongly and positively correlated ( $P \leq 0.01$ ) with grain yield per unit area, and also with average individual kernel mass ( $p < 0.05$ ). Nevertheless, its correlation with number of kernels per three plants in a pot was not significant. On the other hand, contrary to this observation, the average starch content ( $\text{mg seed}^{-1}$ ) was strongly but negatively correlated ( $P \leq 0.01$ ) with number of kernels per pot, but positively with average single kernel mass. Its correlation with grain yield per unit area was not significant at the 5 % probability level.

It, therefore, appeared that the starch content in a given mass of seeds was more associated with average kernel mass, and grain yield per unit area, while the starch content in a single seed was more associated with number of kernels per three plants in a pot and average kernel mass.

**Table 6.14:** Correlation coefficients and significance of the correlation of starch content with some grain yield parameters.

	Starch content (g g <sup>-1</sup> FW)	Starch content (mg seed <sup>-1</sup> )
Starch content (mg seed <sup>-1</sup> )	0.60**	
Average single kernel weight	0.38*	0.84**
Number of kernels per three plants	0.16 <sup>ns</sup>	- 0.46**
Grain yield (g mt <sup>-2</sup> )	0.47**	0.12 <sup>ns</sup>

\*,\*\* = correlation significant at the 5% and 1% probability levels respectively.

ns = correlation not significant at the 5 % probability level.

### 6.3.9 Statistical analysis of the effect of cultivar X ComCat treatment interaction on the starch content in seeds of two Ethiopian wheat cultivars

In this interaction (values pooled for both levels of water treatment) the application of ComCat tended to enhance starch content as expressed both on a g g<sup>-1</sup> FW and mg seed<sup>-1</sup> basis in the cultivar HAR-2508 while it showed no consistent trend in the cultivar ET-13 (Table 6.15). In the former cultivar, the double application of ComCat (CC-13 & 19) yielded starch content (g g<sup>-1</sup> FW) that was +12%, +14%, and +7% higher than that of CC-0, CC-13 and CC-13&19 treatments, respectively. However, according to the analysis of variance, only the first two of these differences were

statistically significant ( $P < 0.05$ ). On the other hand, the starch content ( $\text{g g}^{-1}$  FW) appeared to be slightly higher in the cultivar ET-13 compared to that in HAR-2508, while the reverse was true in starch content expressed on a  $\text{mg seed}^{-1}$  basis (Table 6.15).

**Table 6.15:** Influences of cultivar X ComCat treatment interaction on starch content in seeds of two Ethiopian wheat cultivars, as expressed both on a  $\text{g g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis.

ComCat treatments	Starch content ( $\text{g g}^{-1}$ FW)		Starch content ( $\text{mg seed}^{-1}$ )	
	HAR-2508	ET-13	HAR-2508	ET-13
CC-0	0.57 c	0.63 AB	25.32 A	21.04 A
CC-13	0.56 c	0.58 BC	25.90 A	20.43 A
CC-19	0.60 ABC	0.63 AB	27.74 A	23.02 A
CC-13&19	0.64 A	0.59 ABC	29.49 A	21.57 A
CV (%)	6.22		10.36	
SE	0.0152		1.0283	

Similar letters in front of mean values indicate non-significant differences at the 5 % probability level, while different letters indicate significant differences.

### 6.3.10 Statistical analysis of the effect of water X ComCat treatment interaction on the starch content in seeds of two Ethiopian wheat cultivars

The interaction of these two factors (values pooled for both cultivars) was not significant at the 5% probability level in terms of starch content ( $\text{g g}^{-1}$  FW), indicating that ComCat did not show any significant effect under both levels of water treatment

(Table 6.16). However, the interaction was significant ( $P < 0.1$ ) in terms of starch content as expressed on a  $\text{mg seed}^{-1}$  basis (Table 6.16).

**Table 6.16:** Influences of cultivar X ComCat treatment interaction on starch content in seeds of two Ethiopian wheat cultivars, as expressed both on a  $\text{g g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis.

ComCat treatments	Starch content ( $\text{g g}^{-1}$ FW)		Starch content ( $\text{mg seed}^{-1}$ )	
	NWS	WS	NWS	WS
CC-0	0.61 <sub>A</sub>	0.59 <sub>A</sub>	25.10 <sub>BC</sub>	21.25 <sub>DE</sub>
CC-13	0.61 <sub>A</sub>	0.53 <sub>A</sub>	27.02 <sub>AB</sub>	19.30 <sub>E</sub>
CC-19	0.64 <sub>A</sub>	0.59 <sub>A</sub>	27.47 <sub>AB</sub>	23.29 <sub>CD</sub>
CC-13&19	0.65 <sub>A</sub>	0.58 <sub>A</sub>	29.59 <sub>A</sub>	21.48 <sub>DE</sub>
CV (%)	6.22		10.36	
SE	0.0152		1.0283	

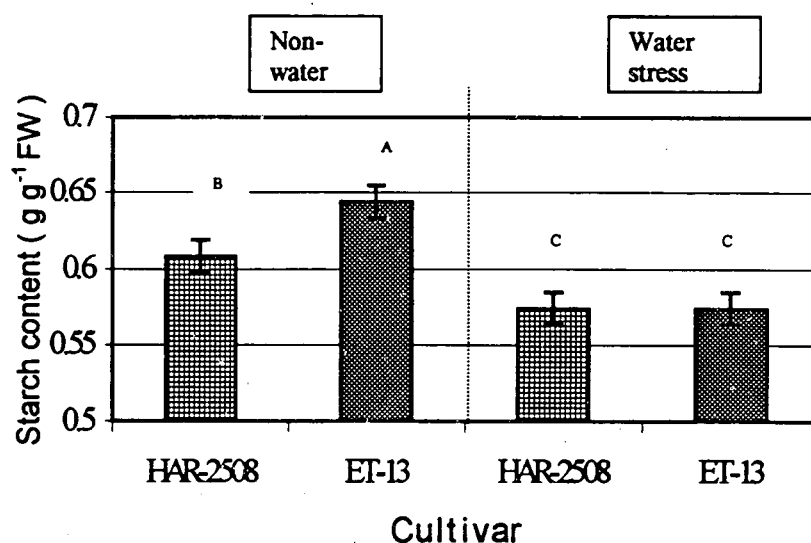
Similar letters in front of mean values indicate non-significant differences at the 5 % probability level, while different letters indicate significant differences.

Starch content ( $\text{mg seed}^{-1}$ ) was higher, albeit non-significantly, in seeds of non water-stressed plants that received the double application of ComCat compared to that of the other ComCat treatments, but it was significantly higher than that of the untreated controls. Furthermore, starch content on a  $\text{mg seed}^{-1}$  basis tended to be higher under the non-water stress condition compared to that under the water stress condition (Table 6.16) where the interaction effect between the two factors was statistically considered.

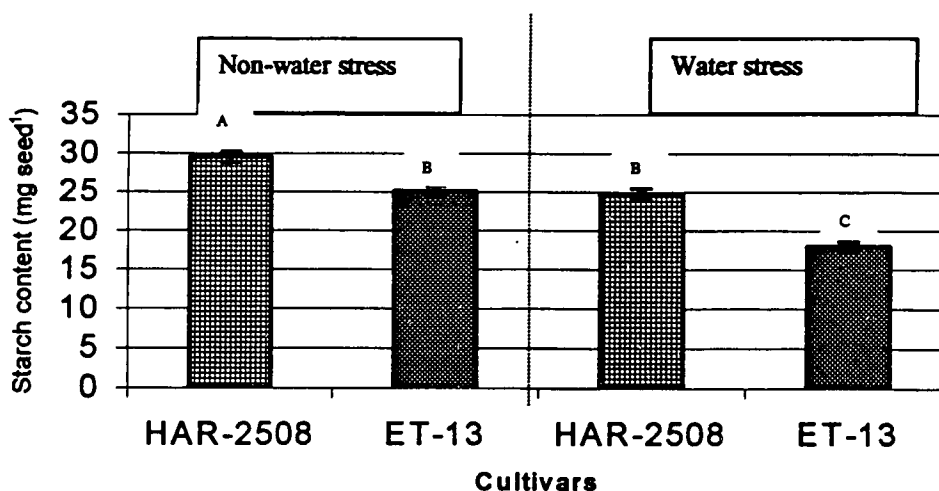
### 6.3.11 Statistical analysis of the effect of cultivar X water treatment interaction on the starch content in seeds of two Ethiopian wheat cultivars

The interaction of the two factors (values pooled for ComCat treatments) was statistically significant ( $P \leq 0.1$ ) under NWS conditions as expressed both on a  $\text{g g}^{-1}$  FW (Figure 6.7) and  $\text{mg seed}^{-1}$  (Figure 6.8) basis. Although seeds of the cultivar ET-13 were significantly superior to those of HAR-2508 in starch content expressed on a  $\text{g g}^{-1}$  FW basis under the non water stress condition, this was not the case under the water stress condition (Figure 6.7).

Although the starch content was reduced in both cultivars because of the water stress, the degree of reduction was different being more pronounced in ET-13 (-12%) than in HAR-2508 (-6%), as expressed on a  $\text{g g}^{-1}$  FW basis. The reduction in starch content followed the same trend when expressed on a  $\text{mg seed}^{-1}$  basis being -19% and -40% in HAR-2508 and ET-13, respectively. This indicated that ET-13 was more sensitive to water stress than HAR-2508 when the effect was measured in terms of starch content.



**Figure 6.7 :** Effect of water X Cultivar interaction on the starch content in seeds of two Ethiopian wheat cultivars as expressed on a  $\text{g g}^{-1}$  FW basis. (Different letters on top of the columns indicate significant difference ( $P \leq 0.1$ ), while similar letters indicate non-significant difference;  $CV=6.22\%$ )

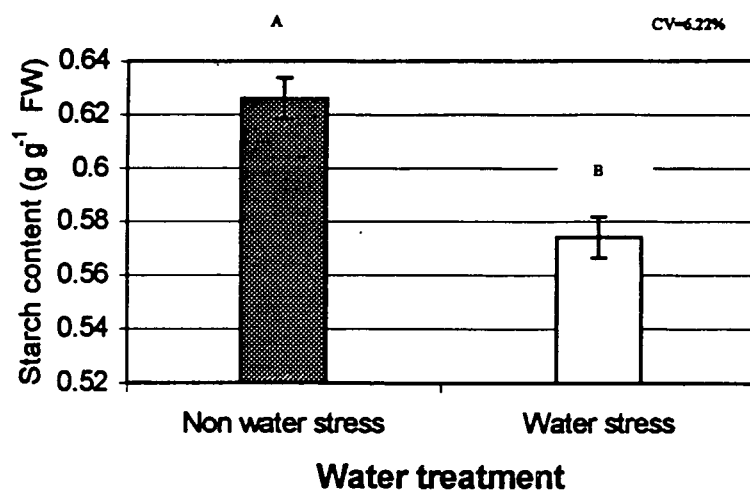


**Figure 6.8:** Effects of water X cultivar interaction on the starch content in seeds of two Ethiopian wheat cultivars as expressed on a mg seed<sup>-1</sup> basis. (Different letters on top of the columns indicate significant difference ( $P \leq 0.1$ ), while similar letters indicate non-significant difference. CV = 10.36).

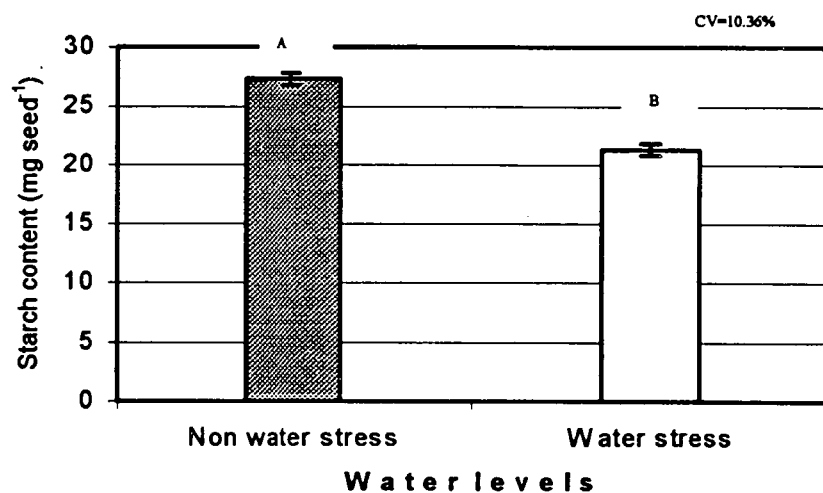
### 6.3.12 Statistical analysis of the individual effects of cultivar differences, ComCat as well as post anthesis water stress treatments on the starch content in seeds of two Ethiopian wheat cultivars

#### 6.3.12.1 Effect of the water treatment

The analysis of variance revealed a highly significant difference ( $P \leq 0.001$ ) in starch content, as expressed both on a g g<sup>-1</sup> FW (Figure 6.9) and mg seed<sup>-1</sup> (Figure 6.10) basis, between seeds from non-water stressed and water deficit stressed plants (values pooled for cultivars and ComCat treatment). The starch content in seeds from plants under NWS conditions were +8% and +22% higher than that in seeds from plants exposed to the water stress treatment, as expressed on a g g<sup>-1</sup> FW and mg seed<sup>-1</sup> basis, respectively. This indicated an inverse relationship between water availability and starch content.



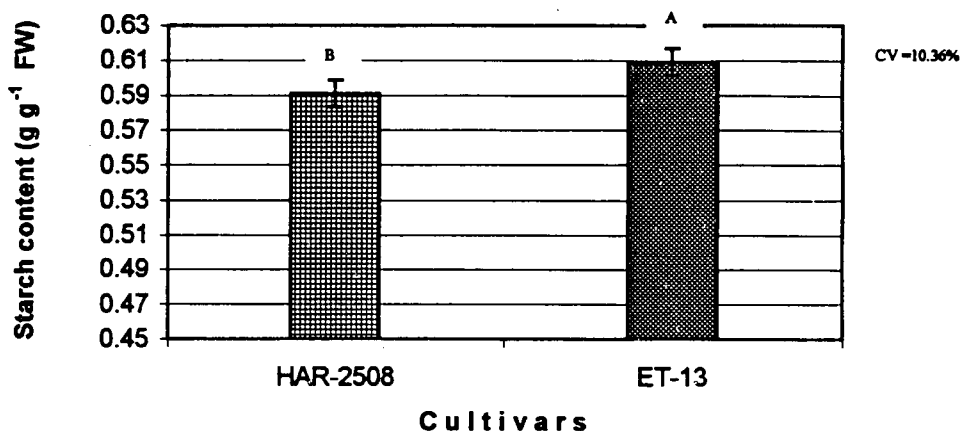
**Figure 6.9:** Starch content in seeds of two Ethiopian wheat cultivars as influenced by water stress treatment and expressed on a g g<sup>-1</sup> FW basis. (Values pooled over cultivars and ComCat treatment). (The different letters on top of the columns indicate significant difference ( $P \leq 0.001$ ),



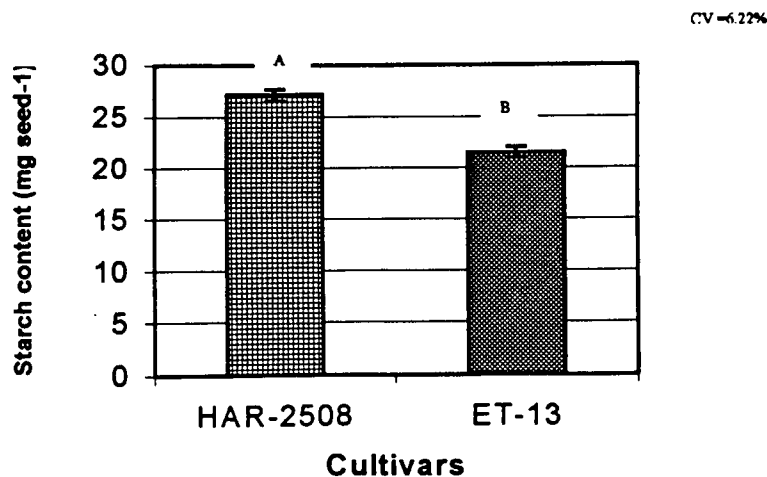
**Figure 6.10:** Starch content in seeds of two Ethiopian wheat cultivars as influenced by a water stress treatment and expressed on a mg seed<sup>-1</sup> basis (values pooled over cultivars and ComCat treatment). (The different letters on top of the columns indicate significant difference ( $P \leq 0.001$ ),

### 6.3.12.2 Effect of cultivar differences

The analysis of variance revealed a significant difference ( $P \leq 0.10$ ) in starch content between seeds of the two cultivars (values pooled for water and ComCat treatments) as expressed both on a  $\text{g g}^{-1}$  FW (Figure 6.11) and  $\text{mg seed}^{-1}$  (Figure 6.12) basis. Seeds from ET-13 showed a 3% higher starch content when expressed in the former manner, while seeds of HAR-2508 had a 21% higher starch content when expressed in the latter manner ( $P < 0.001$ ).



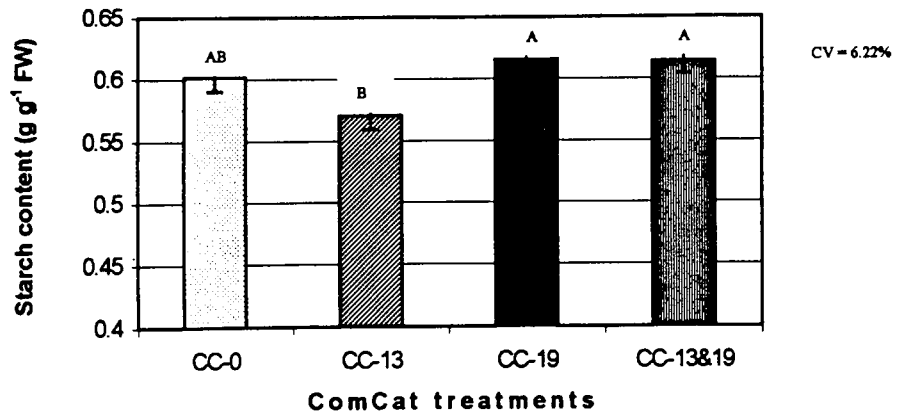
**Figure 6.11:** Starch content in seeds of two Ethiopian wheat cultivars as influenced by cultivar differences and expressed on a  $\text{g g}^{-1}$  FW basis. (Values pooled over water and ComCat treatments). (The different letters on top of the columns indicate significant difference ( $P \leq 0.10$ )).



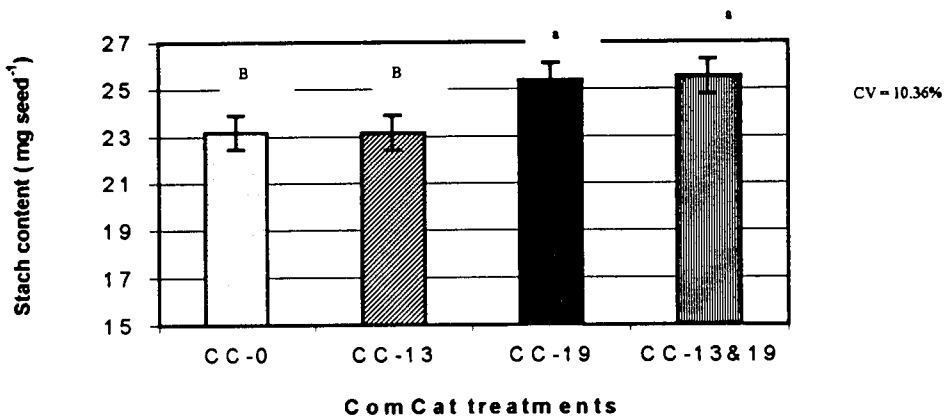
**Figure 6.12:** Starch content in seeds of two Ethiopian wheat cultivars as influenced by cultivar differences and expressed on a mg seed<sup>-1</sup> basis. (Values pooled over water and ComCat treatments). (The different letters on top of the columns indicate significant difference ( $P \leq 0.10$ )),

### 6.3.12.3 Effect of ComCat treatments

Similarly, as was the case with the other main factors, significant differences ( $P < 0.05$ ) in starch content were observed among the four levels of ComCat treatments. The application of ComCat, particularly at later growth stages, tended to increase the starch content, as expressed both on a g g<sup>-1</sup> FW (Figure 6.13) and mg seed<sup>-1</sup> (Figure 6.14) basis.



**Figure 6.13:** Starch content in seeds of two Ethiopian wheat cultivars as influenced by ComCat treatments and expressed on a g g<sup>-1</sup> FW basis (values pooled over cultivars and water treatment). (The different letters on top of the columns indicate significant differences ( $P < 0.05$ )).



**Figure 6.14:** Starch content in seeds of two Ethiopian wheat cultivars as influenced by ComCat treatments and expressed on a mg seed<sup>-1</sup> basis. (Values pooled over cultivars and water treatment). (The different letters on top of the columns indicate significant differences;  $P < 0.05$ ).

## 6.4 Discussion

### Protein content

The protein content of wheat kernels is known to be affected by environmental conditions during growth and by the genetic makeup of the plant, as well as the interaction of these two factors (Donovan *et al.*, 1977; Donovan 1979; Kramer 1979; Fowler *et al.*, 1990; Triboli *et al.*, 2000). This study attempted to investigate the influences of post-anthesis water deficit stress and treatment with ComCat at different growth stages on the protein content in seeds of two Ethiopian wheat cultivars.

The direct interpretation of raw data concerning the influence of the three main factors (water, cultivar and ComCat) revealed that ComCat application at different growth stages had either no appreciable effect or caused a general reduction trend in the WSP content ( $\text{mg g}^{-1}$  FW) in both cultivars and under both conditions of water treatment as compared to the respective untreated controls. WSP content, expressed on a single seed basis, was also generally reduced by ComCat application in both cultivars under both conditions of water treatment (except in the case of CC-13 & 19 treated HAR-2508 plants under the NWS condition). The lack of previous work done specifically on the WSP content in wheat seeds and in relation to ComCat treatments made it difficult to relate these observations.

The water stress treatment *per se*, on the other hand, caused a general increase in the WSP content ( $\text{mg g}^{-1}$  FW) in both cultivars. Some ComCat treatments (CC-13 and CC-19) tended to minimize this trend in seeds from both HAR-2508 and ET-13. However, on a  $\text{mg seed}^{-1}$  basis, a trend to rather decrease the WSP content was evident, particularly in ET-13 plants. Although these variations were statistically non-significant ( $P < 0.05$ ), the trends which were more or less consistent in both cultivars clearly indicated that ComCat had an increasing effect on the water soluble protein content.

On the other hand, the application of ComCat at different growth stages showed an increasing trend in the total protein content (TPC) in both cultivars and under both conditions of water treatment as expressed both on a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis. These observations were in agreement with what was observed in a field study conducted on a South African wheat cultivar over two seasons. In this study the ComCat treatment, particularly when applied at growth stage 13, resulted in a significant increase in the total protein content in wheat seeds (Pretorius J.C. - personal communication).

The water stress treatment *per se* also resulted in a considerable increase in the total seed protein content ( $\text{mg g}^{-1}$  FW) in both cultivars, but was more pronounced in the cultivar ET-13, which is relatively less drought tolerant. The stress condition also caused an increase in the total protein content on a single seed basis in ET-13 plants but showed no consistent pattern in HAR-2508. The increase in total protein content under the influence of water stress was in agreement with results reported by Fowler *et al.* (1990) who reported a negative correlation between water availability and total protein content. However, with regard to both WSP and total protein content in seeds of both cultivars and under both conditions of water treatment, the observed differences were not significant at the 5% probability level.

Further statistical analysis of the effects of the interactions of any two of the three main factors revealed that, although some consistent tendencies were observed, the cultivar X ComCat as well as the water X ComCat treatment interactions were not significant at the 5% probability level. However, the analysis revealed a statistically significant interaction between water treatment and cultivars (data pooled for ComCat treatments) at the 5% probability level in terms WSP and total seed protein content as expressed on both a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis. The general observed trend was that water stress caused a significant increase in these proteins in both cultivars. However, the degree of increase was different, with the higher increase being in ET-13 than that in HAR-2508. This was consistent with the trend observed in the interpretation of the raw data, where protein levels tended to

increase more in the drought sensitive cultivar, ET-13, under the water stress condition.

On the other hand, statistical analysis of the water X cultivar interaction did not reveal any significant differences between water-stressed and non-stressed treatments in terms of the levels of these proteins, as expressed on a mg seed<sup>-1</sup> basis. This indicated that the weight of individual kernels rather than the number of kernels produced per plant, played a more important role in the determination of the final protein levels on a single seed basis. In relation to this, the results have shown that both WSP and total protein content in a single seed were strongly but negatively correlated with the number of kernels per three plants grown in a pot ( $r = -0.57^{**}$  and  $r = -0.90^{**}$  respectively).

The increase in protein content, due to water stress, was more clearly observed from data obtained by means of the analysis of the individual effects of water treatment (data pooled over cultivars and ComCat treatments). The statistical analysis revealed a 19% and 26% increase in WSP and total protein content in a gram of seed, respectively, under the influence of the water stress treatment. This observation of an increase in protein content due to water stress was in agreement with other reports. Stewart and Dwyer (1990) and Fowler *et al.*, (1990) observed negative correlation between the drought effect and protein content in wheat seeds. In this study (see chapter 5), a significantly higher grain yield under non-water stress conditions was measured compared to that under the water stress condition.

However, although the protein content (expressed on a mg g<sup>-1</sup> FW basis) increased under the water stress condition in terms of total protein yield per unit area, the total protein yield under the non-water stress condition was still highest. Based on the findings in this study, the total protein yield per unit area was 8% higher in seeds from non-water stressed plants than that from water stressed plants. This is also in agreement with the findings of Grant *et al.* (1991) who reported a decrease in seed protein content but an increase in total protein yield in barley as moisture supply increased. Another possible explanation for the increase in total protein content

could be an increase in the prolamine fraction of protein, to which the amino acid Proline belongs. A significant increase in this particular amino acid during drought stress is commonly observed in cereals (Munns *et al.*, 1979; Turner, 1997).

Furthermore, the statistical analysis of the individual effect of cultivar differences revealed significant differences between the two cultivars (data pooled over water and ComCat treatments) in terms of protein content in seeds. Seeds of the cultivar HAR-2508 showed a significantly higher ( $P < 0.01$ ) WSP content (expressed on a  $\text{mg seed}^{-1}$  basis) as well as higher total protein content as expressed on both a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis. However, seeds of the cultivar ET-13, by virtue of its higher grain yielding ability (i.e.  $534 \text{ g m}^{-2}$ ), had a 30% higher protein yield per unit area than HAR-2508, whose grain yield was  $409.4 \text{ g m}^{-2}$ . This difference between the two cultivars appeared to be solely associated with differences in their genetic make up. Fowler *et al.* (1990) and Motzo *et al.* (1996) attributed similar variations in protein content observed in other wheat cultivars to genetic differences. Moreover, according to Higgins (1984), the synthesis and accumulation of storage proteins in wheat grains is principally genetically controlled.

Statistical analysis of the individual effect of the third important factor, namely the ComCat treatment (i.e. data pooled over both cultivars and water treatments), shed some light on its important role in terms of seed protein content. Although the identification of the exact mechanism involved was beyond the scope of this study, the application of ComCat appeared to have interfered with protein metabolism in one or other way. Significant differences among the levels of ComCat treatments were observed in terms of seed protein content. The WSP content ( $\text{mg g}^{-1}$  FW) was highest in seeds obtained from untreated control plants. However, it was only significantly higher than in seeds from CC-13 treated plants and it was not different from CC-19 and CC-13 & 19 treated ones. This indicates that the application of ComCat did not increase the level of WSP in seeds. To the contrary, the application of ComCat, particularly at the early growth stage (i.e. CC-13), increased the total seed protein content, as expressed both on a  $\text{mg g}^{-1}$  FW ( $P < 0.10$ ) and  $\text{mg seed}^{-1}$  ( $P < 0.05$ ) basis. The significant increase in total protein content in seeds by the

ComCat treatment may have been related to an increase in specific proteins that are known to be associated with drought stress. Thus, further investigations seem to be essential to identify the types of proteins affected, as well as the exact mechanisms involved. Particular focus may be placed on the prolamines group, where the amino acid Proline belongs.

### Starch content

Interpretation of the raw data revealed that the application of ComCat at different growth stages tended to slightly enhance the starch content in seeds, as expressed both on a  $\text{g g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis, in both cultivars and under both conditions of water treatment. The only exception was the seed starch content in the cultivar ET-13 under water stress conditions. It also appeared that the earliest application of ComCat (CC-13) did not enhance the starch content in seeds. However, the lack of previous reports in the literature on the effects of ComCat treatment on starch content in wheat grains did not allow for verification of the findings in this study.

Further statistical analysis of the interactions of any two of the three main factors also showed some significant effects. In this regard the cultivar X ComCat treatment interaction (data pooled over both water treatments) showed significant differences in seed starch content as expressed on a  $\text{g g}^{-1}$  FW basis. However, a slightly higher starch content was measured in seeds of the cultivar ET-13 compared to that in seeds of HAR-2508. This observation also appeared to be related to the grain yield per unit area. The correlation analysis indicated that starch content, as expressed on a  $\text{g g}^{-1}$  FW basis was positively and significantly correlated ( $r = 0.47^{**}$ ) to grain yield per unit area, indicating that the cultivar with a higher grain yield had a higher starch content in seeds and this was especially true for the cultivar ET-13. With regard to seed starch content expressed on a  $\text{mg seed}^{-1}$  basis, the observed trend, albeit non-significant, was that the starch content was relatively higher in seeds of

the cultivar HAR-2508 and this appeared to be related to the number of kernels produced per plant. The correlation analysis revealed significant but negative correlation between starch content, expressed on a  $\text{mg seed}^{-1}$  basis, and the number of kernels per plant. This indicated that the cultivar with the lower number of kernels per plant, namely HAR-2508, contained more starch content per seed.

On the other hand, statistical analysis of the water X ComCat treatment interaction revealed no significant differences in seed starch content, as expressed on a  $\text{g g}^{-1}$  FW basis, while it was significant on a  $\text{mg seed}^{-1}$  basis at the 5% probability level. However, in both cases the seed starch content tended to be reduced under water stress conditions.

Analysis of the cultivar X water treatment interaction in terms of starch content, revealed significant differences when expressed on both a  $\text{g g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis. Starch content in seeds of both cultivars were reduced due to the water stress, with the highest reductions being in the cultivar ET-13. A similar, but more pronounced effect of water stress on starch content was observed in the analysis of the individual effect of water treatment (values pooled over both cultivars and ComCat treatment). Reductions in starch content of -8% and -22%, as expressed both on a  $\text{g g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis, respectively, were observed.

These observations appeared to be in agreement with other reports. According to Baruch *et al.* (1979) as well as Pannozo and Eagles (1998), the synthesis and accumulation of starch in wheat grains are greatly influenced by environmental conditions, particularly during the post anthesis period. The effect of water stress appeared to be related to its effects on the starch synthesis pathway. Sucrose is reported to be the major carbon source for starch synthesis and is transported to the developing grains from leaves where it is formed (Preiss, 1982). One mechanism by which the water stress treatment reduced the starch content could have been through limiting the supply of assimilates to the grains. Another possible explanation for the reduction in seed starch content could be through the inactivation of the mechanism of conversion of sucrose to starch. A general increase in sucrose

content and a decrease in starch content due to water stress was reported by Slatyer (1969). The author attributed the reduction in starch content to reduced photosynthesis, increased hydrolysis of existing starch as well as to a decreased rate of synthesis. Furthermore, Geigenberger *et al.* (1997) reported inhibition of incorporation of glucose to starch in potato under water deficit stress. The authors further reported that an increase in the degree of the stress changed the relation between sucrose breakdown and sucrose synthesis, in favour of synthesis. They attributed this effect to a possible inhibition of the activities of one or more of the enzymes involved in the terminal reactions of starch synthesis.

Fisher and Cash-Clark (2000), also reported that drought stress affected the translocation of assimilates to the developing wheat grains by affecting the sieve tube unloading step in the control of assimilate import. Thus, the observed reduction in starch content of wheat seeds exposed to water deficit stress, could be through a reduction in assimilate supply, inhibition of the sucrose-starch conversion pathway or through its effect on the assimilate transport pathway.

The variation in starch content ( $\text{g g}^{-1}$  FW) also appeared to be related to grain yield per unit area. Starch content in a given mass of wheat seed was significantly and positively correlated with grain yield, indicating that the cultivar with a higher grain yield per unit area showed a higher grain starch content expressed on a  $\text{g g}^{-1}$  FW basis. This is consistent with what was stated earlier in the discussion regarding the cultivar X water interaction, where the higher yielding cultivar (ET-13) showed higher grain starch content.

On the other hand, starch content in a single seed strongly but negatively correlated with the number of kernels produced ( $r = -0.46^{**}$ ), and also strongly and positively correlated ( $r = 0.84^{**}$ ) with the average single kernel weight. This shows that the cultivar that produced a lower number of kernels, and at the same time had a higher average single kernel weight, contained more starch as expressed on a  $\text{mg seed}^{-1}$  basis. Both these conditions were found in seeds of the cultivar HAR-2508. Such

differences in grain starch content between different wheat cultivars were attributed to differences in genetic makeup by Clark *et al.* (1981).

With regard to the third main factor, namely ComCat, this study provided some contradicting information on the influence the biocatalyst had on seed starch content (values pooled over water and cultivars). For instance, the application of ComCat at the later growth stages resulted in increases in seed starch content as expressed on a  $\text{g g}^{-1}$  FW basis. Moreover, the application of ComCat at the early growth stage (i.e. CC-13) resulted in the lowest seed starch content, while the CC-19 treatment yielded the highest starch content, though it was not significantly different from the control and also from the CC-13 & 19 treatment. However, when seed starch content was expressed on a  $\text{mg seed}^{-1}$  basis, the lowest value was measured in the untreated control (CC-0) and the highest in seeds from plants that received the double treatment (CC-13 & 19) while all the other ComCat treatments also resulted in higher seed starch content.

In summary, this study indicated that under water deficit stress, both protein and starch metabolism were affected, more or less in a contrasting manner, resulting in various differences between seeds from water-stressed and non-stressed plants. While the total protein content tended to increase, the starch content was reduced under the water stress condition. The two cultivars also exhibited different responses to the treatments they were exposed to. The cultivar HAR-2508, which was relatively more drought tolerant, was found superior in total protein content ( $\text{mg g}^{-1}$  FW), while the more sensitive cultivar ET-13 excelled in starch content ( $\text{g g}^{-1}$  FW). The study also showed that treatment with ComCat significantly and positively influenced both protein and starch metabolism over and above to the positive effect on root development, that was dealt with in the preceding chapters.

## Chapter 7

### General discussion

Wheat crops growing in the field under rainfed conditions, more often than not face drought stress of different magnitudes at one or other stage of development (Saini and Westgate, 2000) and this has an impact on the final grain yield and quality. This study aimed to investigate, under glass house conditions, the growth performances of two Ethiopian bread wheat cultivars under the influences of imposed post anthesis water deficit stress and treatment with ComCat, a biocatalyst of plant origin. The study also investigated the possible role of ComCat in circumventing the effects of post anthesis water deficit stress as well as its effects on pre- and post-anthesis growth and development of the cultivars, including its effects on the seed protein and starch contents.

The direct interpretation of the data, at the selected four growth stages during the preanthesis growth period, as well as the statistical analysis of cultivar X ComCat treatment interaction and the individual effects of ComCat treatments, all consistently confirmed that the application of ComCat at different growth stages affected root development more than the vegetative development of the above ground parts. This was in agreement with the observations made in trials over two seasons on wheat and maize cultivars in South Africa. The use of ComCat, more particularly the early application (CC-13), resulted in significant increases in root development (Pretorius J.C.- personal communication; results not shown). This observation also agreed with claims made by the manufacturers of the biocatalyst (Agrorum, Germany).

Additionally, treatment with ComCat also had a significant effect on some yield components. In this regard, the interpretation of data revealed that the application of ComCat tended to enhance the number of kernels per spike and per plant in both cultivars. Particularly the statistical analysis of the cultivar X ComCat interaction showed a significant effect ( $P < 0.1$ ) in terms of the number of kernels per three plants grown in a single pot. In this regard, the early ComCat application (CC-13) resulted in

the highest number of kernels in the cultivar ET-13. However, the interpretation of raw data as well as data obtained through statistical analysis failed to show significant effects of the ComCat treatments on the most important yield component, namely grain yield per unit area. The inability of ComCat to increase the grain yield under glass house conditions was contrary to the observations made in field trials by the University of the Free State, South Africa, and also with the claims made by the manufacturers. This is presumed to be because of the restriction imposed on the roots by the rather small pots. Even though root growth was significantly enhanced by the ComCat application, this appeared not to be sufficient to make a difference in grain yield because of the limited soil surface which the roots could benefit from in terms of extra water and nutrient uptake.

ComCat treatment also had some influence on the grain protein and starch contents of the two cultivars. ComCat application at different growth stages had either no appreciable effect or tended to reduce the water soluble protein (WSP) content in seeds, while it tended to increase the total protein content (TPC). On the other hand, the water X ComCat and the cultivar X ComCat interactions were non-significant in terms of both WSP and total protein contents. Further analysis of the individual effects of ComCat treatments also showed no appreciable effect on the WSP content, but particularly the early application (CC-13) increased the TPC in seeds. The latter was in total agreement with the claims made by the manufacturers (Agraforum, Germany), as well as the results obtained during field trials (University of the Free State, South Africa, research station). As the total protein fraction of wheat grains comprises a range of different protein species, it seems appropriate to further identify the specific proteins affected by the ComCat treatment. In this regard, and based on the claims that ComCat has an enhancing effect on the plants' own defense mechanisms, it might be appropriate to presume that the biocatalyst might have had a similar influence on the pathogenesis related (PR) proteins claimed by Roth *et al* (2000). Moreover, it might be worthwhile to also follow the possible induction of the prolamine group of proteins, where the amino acid proline belongs. The latter is known to be significantly increased during stresses, including drought stress (Munnes *et al.*, 1979; Turner, 1997).

With regard to the effect of ComCat on grain starch content, it was observed that, albeit non-significantly, the ComCat application tended to increase the seed starch content. Furthermore, statistical analysis of the individual effects of the ComCat treatments revealed that the seed starch content was increased particularly by the application of ComCat at later growth stages (CC-19 and CC-13 & 19). Unfortunately, the lack of previous reports in the literature, specifically addressing the effect of ComCat treatment on seed starch content, made it difficult to verify these observations.

The other main variable considered in this study was the water stress treatment which commenced when the plants reached growth stage 60 (beginning of anthesis). Measurements taken during subsequent growth stages consistently revealed that the stress condition affected vegetative growth and yield components as well as grain protein and starch contents in seeds of both cultivars under scrutiny. At growth stages 73-77 it was already clear that the water stress treatment that lasted for two weeks had a significant effect on vegetative growth in terms of all growth parameters except plant height. The lack of a significant difference between the heights of well watered and water stressed plants appeared to be in agreement with reports by Clark and Durley (1981). The authors stated that anthesis and fertilization mark the final stages of vegetative growth in wheat and other cereals, hence no significant height increment could be expected after anthesis whether or not there is water stress.

On the other hand, no significant differences were observed between well watered and stressed plants in terms of the only reproductive parameter measured at growth stages 73-77, i.e. the dry mass of spikes. A possible explanation for this effect seem to lie in the reports of Munns *et al.* (1979), Barlow *et al.* (1980) and McDonald and Davies (1996) who stated that the apical meristems of wheat are capable of surviving severe water stress because of the anatomical protection provided by the surrounding leaf sheaths and the ability of the apex to osmoregulate. Water stress had severe effects on all yield components as well as on the mass of air-dry above ground plant parts as measured at harvest. The most important yield component, grain yield per unit area, was also significantly reduced by the water stress. In most of these cases, the severe

effects of the stress were alleviated by some of the ComCat treatments. These alleviating effects of ComCat are in agreement with the claims made by the manufacturers (Agraforum, Germany).

Furthermore, the statistical analysis of the water X cultivar interaction revealed a significant ( $P < 0.05$ ) interaction effect in terms of each of the protein parameters (i.e. the water soluble and total protein content in seeds). The water deficit stress caused a significant increase in the WSP and total protein content in seeds of both cultivars, but more in ET-13, which is more drought susceptible than HAR-2508. The increase, particularly of the total seed protein content, was in agreement with reports by Fowler *et al.* (1990) as well as Stewart and Dwyer, (1990) who reported a negative correlation between protein levels in wheat grains and the availability of water. Another possible explanation for the increase in seed protein content could be the increase in the prolamine fraction of proteins, to which the amino acid proline belongs. Considerable increases in the proline levels during drought stress were reported by different authors (Munns *et al.*, 1979; Fowler *et al.* 1990; Stewart and Dwyer, 1990; Turner, 1997). ComCat treatments at different growth stages, but especially the early application (CC-13), increased the total seed protein content. However, the mechanism involved in the latter was beyond the scope of this study.

The water stress condition caused a significant reduction in the starch content of seeds, which was in agreement with previous reports (Baruch *et al.*, 1977; Pannozo and Eagles, 1998). According to the authors environmental conditions, particularly during the post anthesis period, influence the synthesis and accumulation of starch in wheat kernels. Slatyer (1969) attributed the reduction to reduced photosynthesis, increased hydrolysis of existing starch and decreased starch synthesis. Fisher and Cash-Clark (2000) ascribed the reduction to the effect of the stress on the translocation of assimilates to the developing grains by affecting the sieve tube unloading process. Geigenberger *et al.* (1997) working on potato plants exposed to drought stress also reported inactivation of one or more of the enzymes involved in the sucrose-to-starch conversion pathway as a possible responsible factor for the reduction. This could also be a possible explanation for the observed reduction in the

starch content in seeds of the two wheat cultivars due to the water stress. This reducing effect by the water stress was, however, circumvented by treatment with ComCat. As a matter of fact the seed starch content was increased by ComCat treatment, and more particularly by application at later growth stages (CC-19 and CC-13 & 19).

The study also investigated the cultivar differences in relation to vegetative growth, yield components as well as grain protein and starch contents. The two cultivars were growing at more or less similar rates during the early growth stages prior to growth stage 34, during which the only significant differences ( $P < 0.05$ ) observed were in stem thickness and leaf area, at growth stage 19, with HAR-2508 being superior in the first, while ET-13 in the latter parameter. At growth stages 34 and 73-77, the cultivars significantly differed in all parameters that measure size and mass gains with ET-13 being superior to HAR-2508 in most of them. Furthermore, as commonly observed in other wheat cultivars (Clark *et al.*, 1981; Belay *et al.*, 1993), these two Ethiopian wheat cultivars not only showed highly significant differences in their vegetative growth patterns, but also in yield components as measured at harvest. The cultivar ET-13 was significantly ( $P < 0.001$ ) superior to HAR-2508 in terms of most of the yield parameters including number of kernels per spike, total number of kernels per three plants, fresh mass of kernels and grain yield per unit area. The cultivar HAR-2508, on the other hand, was superior to ET-13 ( $P < 0.001$ ) in hundred kernel mass and harvest index. However, according to the statistical analysis of the water X cultivar interaction, comparatively the cultivar HAR-2508 appeared to be less affected by the water stress treatment. The analysis revealed that the water stress treatment consistently and significantly reduced most of the yield components in both cultivars, with higher reductions occurring in the cultivar ET-13. The observed reducing effect of water deficit stress on grain yield was in agreement with reports of several authors (Clark and Durley, 1981; Kobata *et al.*, 1992 and Turner, 1997). The authors ascribed the reduction in grain yield due to water deficit stress to reduction in the photosynthesizing area resulting from the premature senescence of leaves. With regard to the effect of ComCat, as mentioned earlier, no alleviating effect on reduction of grain yield by the biocatalyst was observed.

The statistical analysis also revealed highly significant differences between the two wheat cultivars, with HAR-2508 having a significantly ( $P < 0.001$ ) higher WSP and total protein content compared to that of ET-13. Furthermore, when grain protein content variation was analyzed in relation to the water X cultivar interaction, the water stress was found to have had caused a significant increase in both WSP and total protein content in both cultivars, but more in seeds of ET-13 plants, which happened to be more drought susceptible. This variation is presumed to be partially related to the protein increasing effect of the ComCat treatment and to the inherent difference in the genetic make up of the cultivars.

On the other hand, the two cultivars also differed in their seed starch contents. The variation in seed starch content ( $\text{g g}^{-1}$  FW) between cultivars correlated statistically with grain yield per unit area. The cultivar with the higher grain yielding ability (in this case ET-13) also showed a higher seed starch content ( $\text{g g}^{-1}$  FW). On the other hand, the variation in starch content between the cultivars ( $\text{mg seed}^{-1}$ ) appeared to be related to the total number of kernels and single kernel weight. The correlation analysis revealed that the seed starch content ( $\text{mg seed}^{-1}$ ) was strongly but negatively correlated with the total number of kernels ( $r = -0.46^{**}$ ) and strongly but positively with the average single kernel weight ( $r = 0.84^{**}$ ). Again it follows that the cultivar producing the lowest number of kernels, but at the same time measured higher in terms of single kernel weight (in this case the cultivar HAR-2508), had a higher seed starch content ( $\text{mg seed}^{-1}$ ). Clark *et al* (1981) ascribed such differences between wheat cultivars principally to differences in genetic makeup.

In summary, the observations made in this study indicated that the two Ethiopian wheat cultivars differed significantly in their vegetative growth patterns, particularly after growth stage 34, but also in terms of their reproductive development. The cultivar ET-13 was superior to HAR-2508 in both vegetative growth and grain yielding ability under both NWS and WS conditions. However, in relative terms, the drought stress more severely affected the cultivar ET-13 than it did HAR-2508 in terms of both

vegetative growth and yield parameters. The latter cultivar, however, was lower in its yielding ability compared to ET-13. But, by virtue of its shorter life span HAR-2508 appeared to have better escaped the damaging effects of the post anthesis water deficit stress. The two cultivars were also significantly different in their protein and starch contents. The cultivar ET-13 was superior to HAR-2508 in starch content per gram of seed ( $P < 0.1$ ) while the opposite was true in terms of starch content per single seed ( $P < 0.1$ ). Similarly, HAR-2508 was superior to ET-13 in terms of total protein content (expressed on both a  $\text{mg g}^{-1}$  FW and  $\text{mg seed}^{-1}$  basis;  $P < 0.1$ ) also in terms of seed WSP content ( $\text{mg seed}^{-1}$ ).

The investigation of the possible role of ComCat in circumventing the damaging effects of the water stress revealed that the biocatalyst showed promising results and the effects might have been related to its enhancing effect on root development, and also to its inherent effect of strengthening the plants' own defense mechanisms to the stress, which presumably resulted from its improvement of on seed protein content. Both these effects of ComCat are consistent with the claims made by the manufacturers of the biocatalyst as well as with the observations obtained from field trials mentioned earlier. It is, however, recommended that the effect of ComCat in alleviating the damaging effects of post anthesis drought stress be investigated under field conditions in Ethiopia in a follow-up study with Ethiopian wheat cultivars.

## Summary and Conclusion

The situation in Ethiopia, where post anthesis water stress caused by early cessation of rainfall is a serious constraint to crop production, supplied the rationale to study the performances of two Ethiopian bread wheat cultivars under induced post-anthesis water deficit stress. The study also involved the investigation of the possible role of ComCat in circumventing the effects of the stress condition as well as its effects on pre- and post-anthesis growth and development of the cultivars, including its effects on the protein and starch contents in seed. The two Ethiopian bread wheat cultivars (HAR-2508 and ET-13) were grown under glass house conditions and subject to post anthesis water deficit stress while the effects of treatment with ComCat, a biocatalyst from plant origin, were followed during the vegetative growth phase as well as at harvest.

Observations made through the different growth stages indicated that the two cultivars were growing at more or less similar rates during the early growth stages after which significant differences between cultivars were observed during later growth stages with ET-13 being superior to HAR-2508 in terms of most of the vegetative as well as yield parameters under both non-water stress and stress conditions, including the most important yield parameter, i.e. grain yield per unit area. The cultivars also showed significant differences in their grain protein and starch contents. The cultivar ET-13 was superior to HAR-2508 in seed starch content expressed as  $\text{g g}^{-1}$  FW while the latter was superior in terms of starch content expressed on a  $\text{mg seed}^{-1}$  basis. On the other hand, seeds of HAR-2508 were superior to those of ET-13 in total protein content (expressed on both a  $\text{mg g}^{-1}$  and  $\text{mg seed}^{-1}$  basis) as well as in water soluble protein content ( $\text{mg seed}^{-1}$ ).

Moreover, the water stress condition induced at growth stage 60 caused significant reductions in vegetative growth and yield components in both cultivars, with the reductions being more pronounced in the cultivar ET-13. On the other hand, the

ComCat treatments at different growth stages tended to enhance root development and tended to circumvent the negative effects the water stress treatment had on the vegetative growth of the plants. However, ComCat had no significant enhancing effect on the grain yield per unit area, which was in contrast to what was observed in field trials on both wheat and maize as well as to claims made by the manufacturers of the compound (Agrorum, Germany). Lack of a significant effect of ComCat on grain yield under glass house conditions was presumed to be related to the restriction imposed on the growth of roots of the plants by the small sized pots in which the plants grew. The biocatalyst, when applied at early growth stages and particularly at growth stage 13, however, significantly increased the total protein content in seeds of water stressed plants.

Finally, based on the observations made in this study and because of its shorter life cycle, the cultivar HAR-2508 seems to be a better choice to ET-13 in wheat growing areas of Ethiopia where terminal water stress, particularly through early cessation of rainfall, is prevalent. However, full recommendation of this cultivar to such areas require the investigation of its performance under other prevalent limiting factors such as diseases, weeds and bread making qualities. With regard to the biocatalyst, ComCat, although it showed no significant effect in improving grain yield per unit area under glass house conditions, based on the claims of the manufacturers and also on the findings during field trials (results not shown) as well as its significant effect in enhancing root development and increasing protein content of seeds as observed in this study, it appears essential to test it further in field trials under Ethiopian conditions.

## OPSOMMING EN GEVOLGTREKKING

Die situasie in Ethiopië, waar droogtestremming na blomvorming algemeen is as gevolg van die lae reënval gedurende hierdie periode, is 'n ernstige probleem wat gewasproduksie benadeel. Laasgenoemde het dan ook die rasionaal verskaf vir hierdie studie en meer spesifiek die vergelyking van twee Ethiopiese broodkoring cultivars wat na blomvorming aan gesimileerde droogtestremming blootgestel was. Die studie het ook 'n ondersoek na die moontlike rol van ComCat, om die effek van droogtestremming teen te werk, ingesluit asook die invloed daarvan op die groei en ontwikkeling van die twee cultivars gedurende die voor- en nablom groeifases en die effek op die proteïen en styselinhoud van sade. Die twee Ethiopiese broodkoring cultivars (HAR-2508 en ET-13) is onder glashuis toestande gekweek en tydens die nablom fase aan droogtestremming blootgestel terwyl die effek van behandeling met ComCat, 'n natuurlike biokatalis wat uit plantmateriaal vervaardig is, tydens die vegetatiewe groeifase en met oes gemoniteer is.

Die twee cultivars het veral gedurende die vroeë groeifases teen min of meer dieselfde tempo's gegroei waarna betekenisvolle verskille gedurende die latere groeifases waargeneem is. In terme van meeste van die vegetatiewe en oesopbrengs parameters, insluitende oesopbrengs per oppervlak eenheid, het ET-13 beter as HAR-2508 presteer onder beide kontrole- en droogtestremmingstoestande. Betekenisvolle verskille tussen die twee cultivars ten opsigte van die proteïen en styselinhoud van saad is ook waargeneem. 'n Hoër styselinhoud, uitgedruk as  $g\ g^{-1}$  vars massa, is in sade van die cultivar ET-13 gemeet maar die styselinhoud was weer hoër in sade van HAR-2508 toe die inhoud as  $mg\ saad^{-1}$  uitgedruk was. Andersyds is 'n hoër totale proteïeninhoud (uitgedruk as beide  $g\ g^{-1}$  vars massa en  $mg\ saad^{-1}$ ) asook 'n hoër wateroplosbare proteïeninhoud ( $mg\ saad^{-1}$ ) in sade van HAR-2508 gemeet.

Die droogtestremmingsbehandeling op groeistadium 60 het betekenisvolle reduksies in terme van beide vegetatiewe groei en oesopbrengsparameters in albei cultivars teweeggebring maar die reduksies was meer geaksentueer in HAR-2508. Die ComCat behandelings by verskillende groeistadia het wortelontwikkeling geïnduseer en ook die neiging getoon om die negatiewe invloede van die droogtestremmingsbehandeling op vegetatiewe groei in 'n mate teë te werk. Maar, ComCat het nie die finale oesopbrengs per oppervlak eenheid verhoog nie en dit was in kontras met die bevindinge op beide koring en mielies onder veldtoestande asook die aanspraak in hierdie verband deur die vervaardigers van die produk (Agraforum, Duitsland). Die onvermoë van ComCat om onder glashuistoestande tot oesopbrengsverhoging aanleiding te gee word toegeskryf aan die beperkinge wat op die plante deur die relatief klein potte geplaas is. ComCat behandeling, en veral die vroeë behandeling op groeistadium 13, het egter aanleiding gegee tot 'n betekenisvolle verhoging van die totale proteïeninhoud in sade van plante wat aan droogtestremming blootgestel was.

Ten slotte, gebaseer op die waarnemings tydens hierdie studie asook vanweë die korter lewensiklus, blyk dit dat die cultivar HAR-2508 aanbeveel behoort te word vir verbouing in die streke van Ethiopië waar droogtestremming teen die einde van die groeisiklus van koring aan die orde van die dag is. Maar, vir laasgenoemde aanbeveling om op volledigheid aanspraak te maak behoort ander aspekte soos prestasie onder die invloed van ander beperkende faktore naamlik siektes en onkruid, asook bakkwaliteit in aanmerking geneem word. Alhoewel ComCat behandeling nie noemenswaardige oesopbrengsverhoging onder glashuistoestande teweeggebring het nie, behoort die gebruik van hierdie natuurlike biokatalis verder onder Ethiopiese veldtoestande ondersoek te word. Laasgenoemde aanbeveling spruit ook voort uit die aanspraak wat in hierdie verband deur die vervaardigers van die produk gemaak is en is ook gebaseer op die bevindinge wat bekom is tydens veldproewe (resultate nie getoon).

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