Effect of drought stress on photosynthetic pigments, osmolytes and antioxidant enzymes in coriander (*Coriandrum sativum* L.)

Bhuvaneswari, R

(13PBO002)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the

Degree of Master of Science in Botany

March 2015

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Signature of the
Supervisor

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CHAPTER – I

INTRODUCTION

Coriander (*Coriandrum sativum* L.) which belongs to the family Apiaceae (Umbelliferae) is a annual herb which possesses nutritional and medicinal properties, besides, it is one of the most commonly used species. The first medicinal use of the plant was reported by the ancient Egyptians (Ewase *et al*., 2013). All part of the plants are edible but the fresh leaves and the dried seeds are the most common parts used in cooking (Momin *et al*., 2012). Both leaves and seeds of the plant are used for medicinal purpose (Ewase *et al*., 2013). In the Indian traditional medicine, coriander is used in disorders of digestive, respiratory and urinary system, as it has diaphoretic, diuretic, carminative and stimulant (Momin *et al*., 2012).

It is extensively grown in Bangladesh, India, Russia, central Europe and Morocco and has been cultivated since human antiquity (Anonymous, 1950). In India, the major coriander growing area lies under semi- arid climate of Rajasthan and Gujarat and North Madhya Pradesh where the crop is cultivated on conserved moisture during rabi season requiring two to three irrigations depending upon soil conditions and rainfall. The area production and productivity of coriander during 2012-2013 in India were 543 thousand metric tons and 1.0 MT /ha respectively (Anon., 2013).

Drought stress is one of the most important environment stresses affecting agricultural productivity around the world and may result in considerable yield reductions (Boyer, 1982., Ludlow and muchow, 1990). Limited water supply is a major environmental constraint for higher productivity of crop plants including coriander. Moisture deficiency induces various physiological and metabolic processes and the adaptability and responses of the plant to water stress depends on duration, magnitude of stress and developmental and differentiation stage of the plant (Kramer and Boyer 1995). Since plants are propagated by seed; germination stage is important and crucial due to an indirect effect on the density of plants. Germination is one of the most important stages in plant growth that is affected by various stresses. Drought tension delays, reduces or prevent germination (oliveria *et al*., 2006).

Drought stress highly affects growth and yield of agricultural crops. Under drought stress various physiological, biochemical and molecular changes occur in plants to thrive under the stress (Arora *et al*, 2002).

The first noticeable effects of drought stress are detected in the smaller leaves, drying up of leaf tips and reduced plant height. At physiological level, reduction in the leaf water potential and osmotic potential occurs. Due to this various adaptational changes occur at biochemical and molecular levels. Among them accumulation of compatible solutes is an effective phenomenon which plays a significant role in improving stress tolerance (Chen *et al*., 2000, Munns, 2005). Of these solutes glycine betaine and proline are two important osmolytes mostly reported under stress in many crops. Along with this, formation of additional proteins under stress have also been reported.

Drought stress causes damage to the pigment system and adversely affects photosynthesis (Rubey, 1998). Drought stress mediates reactive oxygen species (ROS) which damage the plants by causing lipid peroxidation. To counteract and this the plant system produces antioxidants to cope up the damage of ROS and hence adds tolerance to crops to drought stress. Knowledge on drought stress effects on growth, photosynthesis pigments, proteins and antioxidants need to be generated to study the tolerance of coriander towards drought stress.

Since coriander is widely grown and distributed across most arid and semi-arid regions, research to determine the tolerance levels of coriander to drought is necessary. Additionally, a thorough review of literature by the authors has shown the lack of research on Coriander for water stress. Therefore, a pot experiment was designed and conducted to determine the effects of water stress on different Coriander plant parameters. With this view, the present investigation was carried out with the following objectives.

1. To assess the germination % of seeds and under stress.
2. To assess the growth attributes.
3. To quantify the osmolytes such as proline, glycine betaine, and proteins.
4. Electrophoretic separation of proteins.
5. To assess the damage caused by stress to pigment system.
6. To study the antioxidant defense system by analysing the enzymes and their isoforms.

CHAPTER-II

REVIEW OF LITERATURE

The review of literature pertaining to the present study entitled **"Effect of drought stress on photosynthetic pigments, osmolytes and antioxidant enzymes in coriander (*Coriandrum sativum* L.)"** is given below under the following headings.

Coriander

Coriander (*Coriandrum sativum* L.) is an important seed spices crop of family Apiaceae (Umbelliferae) and possess 2n = 22 chromosomes with cross-pollination as mode of reproduction. Western Europe and Asia are considered to be the centre of origin of this crop (Gal *et al*., 2010). Coriander is an annual herbaceous plant extensively grown in India. Its name has been derived from Greek world "Koris" means bed-bug, because of unpleasant, fetid bug like odour of the green unripened fruits (Meena *et al*., 2010). Effect of water stress in coriander seed showed reduction in parameters like total oil, essential oil, test weight and seed size overall changes in quality. (Saxena *et al.,* 2010).

Drought stress

It is one of the important environmental stresses limiting the productivity of crop plants around the world (Bohnert *et al*., 1995). According to Kramer (1980), the worldwide losses in crop yield from drought stress exists the losses from all other causes even a temporary drought can cause substantial less in crop yield (Moseley, 1983). Drought stress decreases the rate of photosynthesis (Kaua mitsu *et al*., 2000) and finally counts upto reduction in crop production also.

Drought impacts includes growth, yield membrane integrity, pigments content osmotic adjustment, water relation and photosynthetic activity (Benjamin and Nielsen 2006). Complete understanding of physio-biochemical responses of plants to drought is needed for improving plant tolerance to drought stress (Jaleel *et al*., 2006) and this has become a major goal in plant breeding research recently (Cattivelli *et al.,* 2008). Drought is one of the most important abiotic stresses that damage the crops and horticultural products worldwide, especially in arid and semi-arid countries (Rajabi and Fetri 2013).

Germination

Germination stage is an important and crucial stage due to its indirect effect on the production of crops. In this stage, soil water acts as an inducer for germination. Hadas (1977) found that water potential in environment is one of the most fundamental parameter for germination. Several studies have showed that a decrease in soil water potential, water uptake is reduced and finally germination capability decreases (Mayer *et al.,* 1989). Reduction in germination was due to changes in biochemical processes under drought (Misra *et al*., 1995). Various respects supporting reduced germination under drought stress have been sustained (Wahid *et al.,* 1997).

Okcu *et al.,* (2005) studied effect of drought stresses on germination and seedling growth of pea. The drought tension had significantly greater inhibitory effect on seed germination, root and shoot lengths characteristics. Inducing drought stress with PEG 6000 and studying germination have been reported by Ashraf *et al*. (2005) and have found that PEG induced drought stress had higher inhibitory effect on seed germination. Okcu *et al*., (2005) have reported that drought stress has greater inhibitory effect on seed germination.

The effect of different levels of drought stress on germination and seedling growth of coriander showed significant reduction in germination in coriander. Studied on the effect of different levels of drought stress on germination seedling growth of coriander and decreased germination percentage with increased stress. Significant reduction in germination percentage with increasing levels of PEG induced drought stress was reported by Rajabi and Fetri (2013). Similar PEG induced drought studies were also done in groundnut and showed reduced germination under stress by Savaliya *et al*. 2014.

Growth attributes

Plant height

Mafakheri *et al*. (2010) studied the effect of drought stress chickpea cultivars and have noted a significant effect on the number of pods and on plant height which showed reduction.

Similarly effect of drought stress on growth and flowering of marigold showed that drought had a highly significant effect on plant height, number of leaves / plant and plant quality (Atif Riaz *et al*., 2013).

Ghamarnia *et al*. (2013) have studied the effect of different water stress regimes on different coriander parameters in a semi-arid climate and have reported that plant height was affected significantly ($P<0.01$) by water stress. A decrease in plant height under water stress was reported in wheat genotypes by Shirazi *et al*. (2014) and in bean cultivar (Emam *et al*., 2010)

Plant weight:

The growth of plants was effected by the drought stress, where drought had highly significant effect on shoot fresh and dry weight, root fresh and dry weight and significant effect on the shoot-root ratio of these weights in Marigold (Riaz *et al* 2013). Reduced shoot, dry weight under drought in coriander was also reported by Rajabi and Fetri (2013). Reduced dry weight of plant in bean was reported by Emam *et al.* (2010).

Biochemical responses

Proline

Varietal differences in drought hardiness have been correlated with the ability to accumulate free proline under water stress conditions in many species including barley (Singh *et al.,* 1972), wheat (Singh *et al.,* 1973) and sorghum (Blum and Ebercon 1976).

An increase of the free proline content in the leaf tissue is noticed in many mesophytic plants during moisture stress .Proline accumulation are favoured by high leaf carbohydrate status and also by illumination. Various experimental methods of water stress imposition demonstrated the accumulation of proline in leaf tissues of young plants (Singh *et al*., 1973., Blum *et al.,* 1976., Hanson *et al*., 1977., Iwai *et al*., 1979., Munns *et al* 1979., Steward 1981., Parameshwara *et al*., 1988).

Under water deficit conditions, the proline accumulation increased several folds in sugarcane and a significant varietal variation was noticed by (Asokan *et al.,* 1978) .Drought resistant varieties accumulated several times more proline than susceptible varieties under drought situations. They suggested that differential accumulation of proline is a useful index in selection of drought resistant types. Free proline accumulation in the water stressed barley leaf blades was reported by (Tulley *et al*., 1979).

Franschine *et al*. (1980) reported that the levels of free proline increased as a consequence of drought treatment in root tips of zea mays and decreased when the seedlings

were rewatered. Marked differences in proline accumulation due to wilting were observed in different maize genotypes (Mukherjee *et al*., 1980). Corso *et al*. (1981) noted in sugarcane that proline was present in greater amount in the drought resistant varieties, which was helpful in the normalization of cellular osmosis.

Paley *et al*.(1981) reported that proline levels of zea mays stem and leaf tissues increased in respone to water stress and the greatest increase occurred in the stem tissues.An appreciable increase in free aminoacid content of leaves as well as root in maize was also reported by Khan and Garg (1981).

The physiological aspects of proline accumulation have been reviewed by Paleg and Aspinall (1981), Hanson (1980), while the biochemical aspects were detailed by Stewart (1981). Drought susceptible varieties of maize produced higher levels of proline than drought resistant varieties (Douglas *et al*., 1981).

Tan and Halloran (1982) Observed cultivar differences in their capacity to accumulate proline in 14 wheat cultivars and this was also inter –correlated with increases in total catabolic aminoacids and sugars during stress.

Morgan *et al. (*1984) suggested that the osmotic constituents contributed to osmoregulation in higher plants under water stress. Increased levels of proline under moisture stress are reported in crops like coffee (Rudolph *et al*., 1986) and Sweet potato (Indira and Kabeerathuma 1986).

Chanan and Ali (1988) showed a high correlation of proline accumulation with stomatal regulation .They concluded that elevated levels of proline under moisture stress might play a role in stomatal regulation.

Generally, the plant accumulates some organic and inorganic solutes in the cytosol to raise osmotic pressure and there by maintain both turgor and the driving gradient for water uptake (Rhodes and Samaras 1994). As an osmolyte, the beneficial roles of proline in conferring osmotolerance have been widely reported (Bajji *et al*., 2000).A marked increase in free proline content normally occurs in leaf tissue of many mesophytic plant during moderate to severe water deficit. Increased proline accumulation was also reported in Brassica juncea cultivars under water stress (Phutela *et al*., 2002).

Under PEG treatment of -1.5 MPa water stress resulted in increased accumulation of proline which was related to protein hydrolysis, an increase in ornithine -s-aminotransferase

activity, an increase in ammonia content ,increase in precursors of proline biosynthesis ,glutamic acid ,ornithine and arginine in rice (Hsu *et al*.,2003).

Under severe drought stress proline accumulation had increased dramatically in sensitive type of soybeans plants (De Ronde *et al*., 2004). Sumithra and Reddy (2004) reported a significant increase in proline content in cowpea seedlings with progressive increase under water stress. Proline and ABA levels in two sunflower genotypes subjected to water stress(Unyayar *et al*.,2004).Suggested that free proline accumulated in water-stressed leaves of many crops.

Increased concentration of proline under stress and its decreased concentration after relief has been reported in sugarcane crop (Tomader *et al*., 2006).These findings suggested that organic solutes accumulated during stress are likely to be utilized for growth following stress termination.

Mafakheri *et al*. (2010) studied the effect of drought stress on proline contents in three chickpea cultivars. Drought stress increased proline content about tenfold, this increasing roles as an osmotic compatible and adjust osmotic potential which resulted in drought stress avoidance in chickpea. Gosavi *et al*. (2014) have carried out research work on chickpea genotypes and have reported significant accumulation of proline in drought tolerant genotypes.

Glycine betaine

Accumulation of glycine betaine in many plants under water stress is thought to be of adaptive value as it is involved in osmoregulation. It protects the enzyme against the destabilization effects of stress (Robert *et al*., 1984) and probably associated with the protection of cellular structures.

Osmotic adjustment allows cell enlargement and plant growth during severe water stress and allows stomata to remain partially open and co_2 assimilation to continue at lower water potentials (Pugnaire *et al*., 1994). Glycine betaine accumulation in many plants which are exposed to various abiotic stresses, has been documented and was positively correlated with stress tolerance (Saneoka *et al*., 1995).

Reports supporting the fact that glycine betaine stabilized the protein of PS II complex and preventing membrane damage from stress by direct membrane stabilization have also been demonstrated (Yang *et al*., 1996).

The physiological significance of co-accumulation remains unclear since glycine-betaine and proline remains may reduce the extent of proline accumulation (Gibon *et al*., 1997). In some plants species, accumulation of glycine-betaine and proline may occur concomitantly (Girija *et al.,* 2002). Glycine betaine accumulation under drought stress under higher plants like onion (Mansour, 1998), rice (Mohanty *et al*., 2002), mustard and mulberry (Ahmed, 2010).

Iqbql *et al.,* (2005) reported influence of water stress and exogenous glycine betaine on sunflower achene weight and oil percentage. Foliar sprays of glycine betaine, however, significantly reduce the negative effects of water stress on achene weight. Higher glycine betaine accumulation potential in cotton genotypes under drought stress has shown to add tolerance in cotton.

Kashif Shahzad *et al*. (2006) reported endogenous glycine betaine accumulation in plants varies from species to species under stress. Significant increased levels of glycine betaine was reported in cotton genotypes under drought stress (Shahzad, *et al*., 2006).

Wang *et al*. 2010 reported that enhancement of antioxidant activity and improvement of water status is due to the accumulation of glycine betaine in wheat. Plants have known to accumulate glycine betaine naturally under drought stress (Alcazar *et al*., 2010) Shahbaz *et al*. (2011) explored the effectiveness of exogenously appealed glycine betaine as foliar spray in mitigating the harmful effect of drought on wheat crop and proved better performance and reduced adverse effects of drought stress on wheat crop.

The efficacy of glycine betaine for drought tolerance in two contrasting maize cultivars to improve growth and productivity under water-stressed conditions was reported by Shakeel *et al*. 2012. Raza *et al.* (2014) have reported that exogenous application of glycine betaine and potassium improved water relations and grain yield of wheat under drought studies similarly and also reported the impact of foliar applied glycine betaine on growth and physiology of wheat under drought condition.

Pigments

Balakumar *et al*. (1988) reported reduction in chlorophyll propotional to carotenoids in cotton and sorghum to moisture stress. Paramasivam *et al*. (2009) have reported photosynthetic pigments and carotenoids show multifarious roles in drought tolerance including light havesting and production from oxidative damage caused by drought.

Mafakheri *et al*. (2010) showed that the chlorophyll a was more sensitive to drought stress than chlorophyll b.

Shamsi *et al*. (2010) studied the effects of drought stress on chlorophyll content in bread wheat cultivars under field conditions. The results showed that with an increase in the intensity of drought stress on wheat cultivars, there was a decrease in total chlorophyll content. Bhosale and Shinde (2011) reported that the amount of chlorophyll content was found to be decreased due to increase in water stress.

Shakeel and Muhammad (2011) carried out studies morphological, physiological and biochemical responses of plant to drought stress. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and maybe the result of pigment photo-oxidation and chlorophyll degradation.

Drought stress responses of wheat genotypes with respect to nutritional quality (Azaz *et al*., 2012) reported that drought resistant cultivars, as compared to sensitive genotypes, maintain higher chlorophyll content under drought stress condition. Semsettin *et al*. (2012) reported that the effect of chl a, chl b, total carotenes showed reductions under stress treatments. Similar reports were obtained in pigmented rice by Chutipaijit *et al*. (2012).

Kumar *et al*. (2013) have showed that the photosynthetic pigment like chlorophyll decreased with increasing drought stress. Similar reports showing decrease photosynthetic pigments under drought stress in lettuce plants was reported by Mohammad *et al*. 2013, Maryam *et al*. 2014.

Antioxidant

Superoxide dismutase (SOD)

Various authors have pointed out that drought and saline stress increase SOD activity (Dhindsa and Matowe, 1981., Dhindsa *et al*., 1982., Gaspar and Dhindsa 1981).

Among the antioxidants, SOD is essential components of defense mechanism in plants under environmental adversity (Bowler *et al*., 1992). Water stress did not influence SOD in sorghum (Zhang and Kirkham, 1996) and wheat (Sairam *et al*., 1998., Sairam and Srivastava, 2001).

The activity decreased with osmotic stress in upland rice (Reddy and Vajranabhaiah 1993) and increased in maize (Jagtap and Bhargava, 1995), and wheat (Dawar *et al* 1998).

Several studies have reported that enhanced stress tolerance has been related to over production of chloroplast SOD (Arisi *et al*., 1998).

The capability of scavenging ROS and reducing their damaging effects may correlate with the drought tolerance in plants (Tsugane *et al* 1999). The protective role of SOD with particular attention to the membrane function seems acceptable, even if the complexity of the involved biochemical pathways does not exclude the influence of other metabolic mechanisms. A reduction in the activity of SOD under drought and heat stress and its rapid increase following rewatering was reported in Kentucky blue grass (Zhaolong and Bingru, 2004).

This dynamic mechanism comprises of antioxidant enzymes, such as superoxide this dismutase SOD, peroxidase, POD etc. and maintaining a relative higher antioxidant activity leads to drought tolerance improving the capacity to cope with ROS (Sharma and Dubey, 2005).

The balance between ROS production and antioxidant enzyme actively determines oxidative signalling (Moller *et al*., 2007). In response to drought, plants have developed a series of enzymatic and non-enzymatic antioxidant system to cope with drought stress (Ali *et al*., 2008).

Mafakheri *et al*., (2010) studied the effect of on antioxidant enzymes and Malondialdehyde content under drought stress in sunflower. Plants under drought stress showed a significant increase and decrease, respectively, in SOD activity in compared to control plants.

Chugh and Kaur., (2011) evaluated the oxidative stress tolerance in maize seedlings in response to drought and superoxide dismutase (SOD) activity was significantly decreased in sensitive genotypes, but remained unchanged in tolerant genotypes under stress.

Ahmad and Haddad (2011) studied the effects of antioxidant enzymes activities and osmotic adjustment of wheat under drought stress. SOD in wheat was increased in the leaves under drought stress. While such an increase was more significant and consistent in si treatment than in other treatments. Esen and Ozgur (2012) reported the antioxidant system responses of a xerophytic plant to drought stress, where SOD activity was increased under drought stress. Similarly increased levels of SOD were observed in barley (Salekjalali and Haddad, 2012) and in Soyabean by Mohamed and Akladious (2014).

Peroxidase (POD)

Under environmental stresses POX enzyme scavenges the ROS and protects the photosynthetic machinery (Sheoran and Garg, 1979; Egert and Tevini, 2002; Rios- Gonzalez *et al*., 2002; Candan and Tarhan, 2003). Under water deficiency conditions O_2 and H_2O_2 increased since the stomata get closed (Larson, 1988). As a result, a significant increase in the content of antioxidant enzymes, like peroxidase was noticed (Larson, 1988., Egert and Tevini. 2002, Yordanov *et al*., 2000). Peroxidases catalyze oxidation of various substrates in the cell (Miller *et al*., 1990) and significant roles of peroxidises have been suggested in plants.

No changes in POX level was noted in wheat (Fangmeiner *et al.,*1994) in response to drought stress. Yang-Li *et al*., (1995) also reported increased POX activity in response to drought treatment in resistant genotypes of sugarcane. Increased POX activity was reported in maize (Zhang *et al*., 1995), decreased in sunflower and sorghum (Zhang and Kirkham, 1996) and increased in grasses (Fu and Huang, 2001), *Cucumis sativus* (Tekechandani and Guruprasad, 1998) wheat (Li and Liang 1988) in response to drought. Similar results of increased oxidative enzymes under drought stress in tolerant wheat genotypes have been reported (Sairam *et al*., 1998).

The peroxidase isozyme banding pattern has been studied in sugarcane clones and occurrence of 19 isozyme bands per cultivar has been reported (Hemaprabha and Sree Rangasamy, 2001). The enzymatic polymorphism in sugarcane cultivars shows that this gene pool is a good resource for breeding. Peroxidase isozyme diversity has been a useful criterion in sugarcane breeding programmes.

Increased activity of POX was reported in cauliflower subjected to drought stress (Chenguoju *et al*., 2002). Vasantha and Rao (2003) reported increased peroxidase activity during stress in sugarcane.

Chugh and Kaur (2011) evaluated of oxidative stress tolerance in maize seedlings in response to drought. Peroxidase (POX) activity was significantly induced in tolerant, as well as sensitive genotypes. Vaziri *et al*., (2011) studied the effect of water deficit on two species of Aeluropus and observed increased POX activity. Significant enhancement of POD activity was reported in wheat (Ahmad and Haddad, 2011).

Esen and Ozgur (2012) determined the antioxidant system responses of a xerophytic plant *Gypshophila aucheri* to drought and reported increased POX activity. The effects of soil water shortages on the activity of antioxidant enzymes in Barley showed significant increases in POX activity in both moderate and severe stress treatments (Salekjalali and Haddad, 2012).

Chugh and Kaur (2013) evaluated the role of oxidative stress management was in two maize and reported increased. POX activity was which influenced both genotypes with increase in drought stress. Similar finding was also reported in soybean (Mohamed and Akladious, 2014).

Protein

Mohhammadkhani *et al.* (2008) studied the effects of drought stress on soluble proteins in two maize cultivars through in roots and leaves and reported that the protein increased first and then decreased.

Abdollah *et al*. (2010) studied the progressive effect water deficit stress on proline accumulation and protein profiles of leaves in chickpea. The SDS-PAGE analysis of soluble proteins from leaves revealed that progressive water deficit stress did not significantly change proteins profile of chickpea cultivars, with the exception that the band intensity of a polypeptide with molecular mass near 150KDa was increased partly in all the cultivar under water stress.

Sharma *et al*. (2013) also supported the formation of proteins under stress by detectable protein bands. Amita *et al*. (2013) reported changes in protein bands in SDS-PAGE gel under drought conditions resistant and susceptible varieties.

The effect of protein profiles and dehydrin accumulation in some soybean varieties in drought stress conditions. The appearance of new protein under PAGE electrophoresis was reported in soybean by Arumingtyas *et al*. (2013). Morad Pour et *al*. (2014) evaluated leaf protein pattern in wheat genotypes under drought stress reported a total number of 35 protein bands detected in leaves under stress.

Isoenzyme

SOD

Antioxidant defense systems are well known for scavenging reactive oxygen species (ROS) produced in different stressful conditions, such as activation of the antioxidant

enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) (Allen *et al*., 1997, Kwon *et al.,* 2002). The scavenging capacity of superoxide radicals (O_2-) is achieved through an upstream enzyme SOD, which catalyses the dismutation of superoxide to hydrogen peroxide (H_2O_2). POD reduces H_2O_2 to water using various substances as electron donors.

The native polyacrylamide gel electrophoresis analysis detected eight SOD isozymes in oilseed rape leaves of three which are isoforms and of Mn-SOD and five isoforms of Cu/Zn-SOD under drought stress (Abedi and Pankniyat, 2010).

POD

Peroxidase (POD) activity increased in the leaf and petiole but decreased in the root under drought in *Ctenanthe setose* (Terzi and Kadioglu, 2006). Five isoforms in rape seed under all drought stress was reported by (Abedi and Pankniyat, 2010).

Sharma *et al*., (2013) studied the expression of boiling-stable peroxidase isoenzymes under combined effect of drought and heat in different tissues of wheat. These result analysis revealed that among various antioxidant enzymes like POD was detected as a boiling stable protein.

CHAPTER – III

MATERIALS AND METHODS

A pot culture experiment was conducted at Avinashilingam University, Coimbatore, TamilNadu, during 2014-2015, utilizing four coriander varieties (*Coriandrum sativum* L.) (*Coriandrum sativum* L.) Such as Local, Ruchi, CO3, CO4. Seeds were collected from Tamil Nadu Agricultural University Coimbatore and sown in 16 pots replicated thrice filled with farm soil and sand (3:1) (Plate 1).

Experiment 1

Determine the germination percentage under PEG induced drought stress

Germination

In order to study the germination and seedling growth of coriander under drought stress, separate experiment was conducted. To create drought stress polyethylene glycol (PEG 6000) was used, respectively. Drought levels included distilled water as a control, 15%, 20%, 25% and 30% w/v of PEG. At first, seeds were disinfected by hypochlorite sodium 2% for 2 min and then were washed with distilled water for 3 numbers and were disinfected by fungicide Benomyl 0.2%. Seeds were washed with distilled water again. A total of 30 seeds were bedded in each pot. The germination was counted and the percentage was calculated.

Experiment 2

To study the physiological and biochemical parameter under drought stress.

Drought treatment

Drought treatment was given by withholding 2 irrigations starting from 15^{th} day to 30^{th} day to the pots allotted for drought treatment. The control pots were given normal irrigation.

Growth Parameter Measurement

In order to measure morphological parameters the plant height and fresh weight were measured.

Plant height

Three shoots from each pot were randomly selected and tagged and the plant height was measured from the base of the shoot up to the tip of leaf. The morphological character of the shoot under stress was also studied.

Plant weight

Three plants from each pot were randomly selected and uprooted and the plant fresh weight was measured at the whole plant fresh out.

Biochemical Parameters

Estimation of Osmolytes

Proline accumulation

The proline content was estimated by the method of Bates *et al*., (1973).

1 g leaf tissue was homogenized with 10 ml of 3% sulphosalysilic acid and filtered through Whatman No.2 filter paper. To an aliquot of 2 ml of filtrate, 2 ml glacial acetic acid and 2 ml of acid ninhydrin reagent (1.5 g ninhydrin dissolved in 20 ml orthophosphoric acid and 30 ml glacial acetic acid) was added and the tubes were incubated in boiling water bath for one hour. The heating was terminated by immediately transferring the tubes to ice bath and after adding 4 ml toluene and the reaction mixture was vortexed to bring the chromophore to the toluene layer, which was separated using separatory funnels. The absorbance of the chromophore was read at 520nm. L- Proline standard was used for quantification and the proline content in the sample was calculated using the formula:

$$\frac{\mu g \text{ proline x ml toluene x } 5}{115.5 \text{ x g sample fr.wt.}} = \mu \text{ mole of proline/ g}$$

Glycine –betaine estimation

For glycine betaine estimation, leaf samples were oven dried at 80^{o}C for 4 days. About 500 mg dried samples were ground to fine powder and shaken at 25^{o}C for 24 hours with 20 ml water. Contents were filtered and stored in a freezer until the analysis was done. Aliquots containing 0.5 ml of thawed extract were diluted to 1:1 with 0.2 N H_2SO_4 and cooled in ice water for 1 hour. Further 0.2 ml cold KI-I_2 reagent (prepared by dissolving 15.7 g of Iodine and 20.0 g of KI in 100 ml water) was added and stirred gently. This was stored at 0- 4^{o}C overnight (16h) and then centrifuged at 16,000 rpm for 15 minutes and supernatant was aspirated with fine tipped glass tube. Throughout the process cold conditions were maintained because the solubility of acid reaction mixture increases markedly with temperature. Periodide crystals formed were separated from the acid media and then dissolved in 9 ml of 1, 2- dichloroethane. Vigorous shaking was done for complete solubilization of crystals. After 2 hours, absorbance was recorded at 365 nm. The amount of

glycine betaine was expressed as mg/g dry weight comparing with glycine – betaine (Sigma) standard (Grattan and Grieve, 1985).

Pigments estimation

1 g of leaf tissue was extracted with 95% ethanol and the filtrate residue was re-extracted with acetone and the volume was made up to 40 ml with acetone. The mixture was transferred into a separatory funnel and 40 ml peroxide free ether was added and the funnel was vigorously shaken so that the solvents were dissolved completely. The pigments were forcibly flushed with distilled water when the ether phase with pigments gets separated on the top and the water settled at the lower side. The water was discarded and the ether phase was collected and finally made upto 40 ml with ether and absorbance was recorded at 665, 649, 642.5, 485, 474 and 470 nm. The pigments were quantified utilizing the following formulae (Weybrew, 1957).

Total chlorophyll	=	5566.5 x A (649)
Chlorophyll a	=	1994.5 x A (665) – 173.4 x A (642.5)
Chlorophyll b	=	3528 x A (642.5) – 607 x A (665)
Total carotenoids	=	982.1 x A (474) – 0.0255x Chlorophyll a – 0.225 x Chlorophyll b
Carotenoids	=	2518.2 x A (485) – 1198.5 x A (470) – 0.0298 x Chlorophyll a + 0.0036 x Chlorophyll b
Xanthophylls	=	2026.1 x A (470) – 2288.6 x A (485) + 0.0036 x Chlorophyll a -0.6518 x Chlorophyll b

The pigment content was expressed in mg g $frwt^{-1}$.

Antioxidants

Peroxidase enzyme activity

Peroxidase activity was assessed following the oxidation of O-dianisidine following the method of Malik and Singh (1980).

1 g of leaf tissue was homogenized in 5 ml of phosphate buffer (pH 7.0) using a pre-cooled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 minutes and the supernatant was saved for further assay. For the assay, 3.5 ml of phosphate buffer (pH

6.5) was taken in a clean dry cuvette and to it 0.2 ml of enzyme extract and 0.1 ml of freshly prepared O-dianisidine solution was added. The assay mixture was brought to room temperature and placed in the spectrophotometer at 430 nm. Then 0.2 ml 0.2 M H_2O_2 was added and immediately the stopwatch was started. Absorbance was read at every 30 seconds interval up to 3 minutes. Increase in absorbance was plotted against time and from the linear phase the change in absorbance per minute was read. Enzyme activity was expressed in terms of rate of increased absorbance per unit time per mg protein or tissue weight. Water was used as a blank for the assay.

3.8.9 Superoxide dismutase activity

Superoxide dismutase (EC 1.15.1.1) was assayed by monitoring the inhibition of photoreduction of nitro blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). Leaf samples were homogenized in four volumes (w/v) of an ice-cold buffer containing 0.1 M Tris-HCl, 1 M EDTA and 0.05% Triton-X 100. The homogenates were filtered through four layers of cheese cloth and centrifuged at 4^{o}C for 30 minutes at 15000 rpm. The supernatant collected was used for SOD assay. The reaction mixture contained 50mM phosphate buffer (pH 7.8), 0.053 mM NBT, 10 mM methionine, 0.053 mM riboflavin and an appropriate aliquot of enzyme extract. The reaction was started by switching on the light and allowing running for 7 minutes. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

Estimation of Protein (Lowery *et al.*, 1951)

Procedure

The blue colour developed by the reduction of phosphomolybdic and phosphotungstic compounds in Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein and the colour developed by biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowery's method.

Chemicals used

1. 2% sodium carbonate in 0.1 N sodium hydroxide (reagent A)
2. 0.5% copper sulphate in 1% potassium sodium tartarate (reagent B)
3. Alkaline copper solution: Mix 50 ml of A and 1 ml of B prior to use (reagent C)
4. Folin-Ciocalteau (reagent D)

5. **Protein solution (stock standard):** Accurately weighed 50 mg of bovine serum albumin and dissolved in distilled water and made up the volume into 50 ml in a standard flask.
6. **Working standard**: Diluted 10 ml of the stock solution to 50 ml with distilled water in a standard flask. 1 ml of the solution contains 200 µg proteins.

Procedure

Extraction is usually carried out with buffers used for enzyme assay. Weighed 1g of the sample and ground well with pestle and mortar using 5-10 ml of the buffer, centrifuged and used the supernatant for protein estimation.

Estimation of Protein

1. Pipetted out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes.
2. Pipetted out 0.1 ml and 0.2 ml of the sample extract into other test tubes.
3. Made up the volume to 1 ml in all the test tubes. A test tube with 1 ml of water served as the blank.
4. 0.5 ml of reagent C was added to each test tube including the blank, mixed well and incubated for 10 minutes.
5. 0.5 ml of reagent D was added, mixed well and incubated at room temperature in dark for 30 minutes. Blue colour is developed and read at 660 nm.
6. The reading at 660 nm was taken. A standard graph was drawn and the amount of protein present in the sample was calculated.

Calculation

The amount of protein in the test sample was expressed in mg /g or 100 g sample.

Electrophoretic separation of proteins

Electrophoresis of protein was carried out through native and SDS-PAGE (Laemmli, 1970). Separation of proteins by native-PAGE depends on both size and charge of protein, whereas SDS-PAGE depends on size. Polyacrylamide gel is formed by polymerizing acrylamide with cross linking agent (bisacrylamide) in the presence of catalyst (persulphate ion) and chain reactor (TEMED). The relative proportion of acrylamide monomer to bisacrylamide determined the porosity of gel. Low percentage of gel (with large pore size) is used to separate high molecular weight protein.

SDS is an anionic detergent, which binds strongly to and denatures proteins on the presence of SDS and reducing agent β-mercaptoethanol; oligomeric proteins are dissociated into a number of amino acid residues in that chain. The SDS-protein complex carries negative charges and hence moves towards the anode and separation is based on the size of the protein.

Sample preparation

Electrophoretic separation of protein was carried out in leaf samples. The leaf material was found to possess better resolution of banding pattern than other parts, and hence was selected for further analysis. The freshly collected leaves were homogenized with phosphate buffer 0.1 M (pH 7) containing α-mercaptoethanol, in the ratio of 1:1 in pre-chilled pestle and mortar. The slurry was centrifuged at 15000 rpm for 20 minutes. The supernatant was collected and aliquots were frozen in small vials.

Preparation of gel

The glass plate, spacers and comb were cleaned thoroughly. The spacers were placed on the edges in between the plates and clamped. The edges were sealed with 1% molten agar. Separating gel without polymerizing agents was prepared (as given below), degassed for 10 minutes in a beaker, mixed with 10% APS and TEMED and quickly poured in between the glass plates.

The gel was overlaid with a layer of water to accelerate polymerization. After polymerization, water was removed and stacking gel was poured over the separating gel and a comb was placed on the top of it. Once polymerization was completed, the set up was mounted to the gel apparatus after removing the clips and holding the glass plate. The tank buffer was poured and the comb was carefully removed to get wells of uniform dimensions.

Native PAGE

The electrophoresis of proteins was carried out using 10% native PAGE (polyacrylamide gel electrophoresis) using 0.25 M tris and 1.95 M glycine, pH 8.3 buffer system (Laemmli, 1970). The protein samples were loaded in the wells of 4% stacking gel and the separation was continued in 10% resolving gel. A constant voltage of 100 V was maintained until the samples reached the resolving gel and then increased to 200 V till the tracking dye reached the bottom of the gel which takes about 4 hours. The separation was carried out at $4^{o}C$.

Isoenzyme pattern of Superoxide dismutase

Superoxide dismutase isoenzyme pattern was obtained by staining the non- denaturing gels (Burk and Oliver, 1992) with minor modifications. Leaf samples were cut and immediately homogenized (1g leaf + 2ml extraction buffer) at 4^{o}C in extraction buffer containing 96 mM Tris-HCl, 13% (v/v) glycerol, 0.6% (w/v) PVPP, 5mM DTT and 1mM PMSF in 1: 10 (w/v) ratio. The extracts were loaded on a non- denaturing (7.5 - 12%) PAGE. Pre-running was performed for 20 minutes at 70 V to remove the ammonium per sulphate. Electrophoresis was carried out at 50 to 60 V at 4^{o}C for 6 hours. Gels were stained by incubating in the dark in 50 mM potassium phosphate buffer (pH 7.5), 0.1 mM EDTA, 0.2% TEMED, 3mM riboflavin and 0.25 mM NBT for 30 minutes at room temperature with constant shaking. After incubation, gels were rinsed and placed in distilled water and exposed to light under 400 WHP sodium lamps, 60cm above for five to ten minutes at 25^{0}C. SOD activity was compared by the intensity and size of the achromatic bands against a blue background.

Statistical Analysis

The data were analyzed by SAS software in a completely randomized design and diagrams were plotted with Excel software.

Plate-1

Experimental setup

R1 of all varities is control

R2-R4 of all varieties is treatment

CHAPTER - IV

RESULTS AND DISCUSSION

Coriander (*Coriandrum sativum* L.) is an important seed spices crop of family Apiaceae (Umbelliferae) and possess 2n = 22 chromosomes with cross-pollination as mode of reproduction. Western Europe and Asia are considered to be the centre of origin of this crop (Gal *et al*., 2010).

Experiment 1

Germination

Germination study for the four varieties selected was carried out by inducing drought stress with varying concentrations of PEG (15%, 20%, 25%, and 30%) (Plate 2).

With the increase of PEG concentration that is the increase in stress, the germination percentage was reduced drastically in all varieties. The varieties showed a maximum reduction at 30% concentration and minimum at 15%. Among the varieties, CO4 and ruchi were effected to a greater extent and showed very high percentage of reduction (20% of germination), compared to the varieties local and CO3. With this result it inferred that drought stress has significant affect on germination in coriander (Table 1 and Figure 1)

Reducing germination components can be attributed to reduction of speed of rate of water absorption and the negative effects of low osmotic potentials and its effects of low osmotic potentials and its effects on biochemical processes of catabolic and anabolic in germination stage (Neto *et al*, 2004).

Significant reduction in germination percentage with increasing levels of PEG induced drought stress was reported by Rajabi and Fetri (2013). Similar PEG induced drought studies were also done in groundnut by Savaliya *et al*. 2014.

Studied on the effect of different levels of drought stress on germination seedling growth of coriander and decreased germination percentage with increased stress (Rajabi and Fetri, 2013).

Plate - 2

Germination

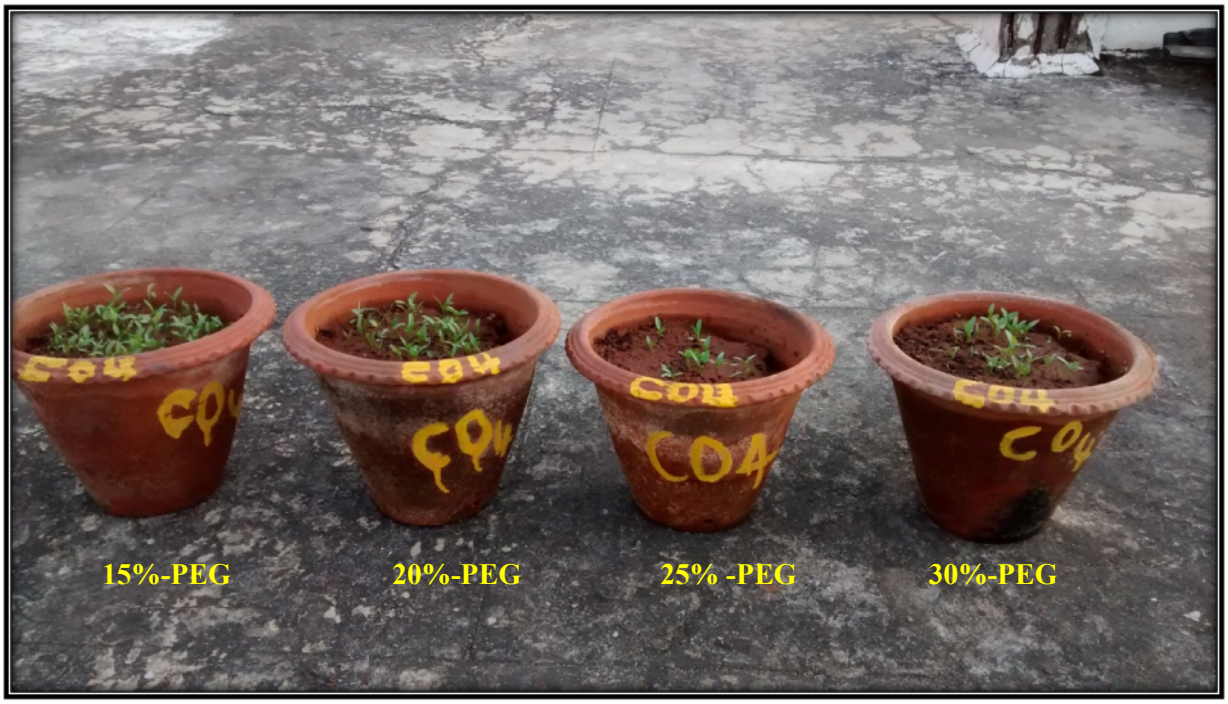


Table - 1

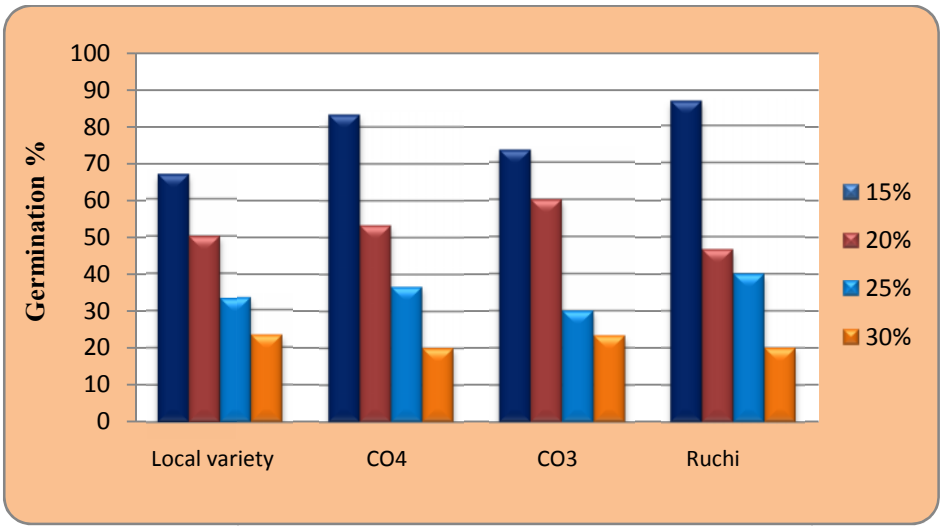
Germination

Concentration	Local Variety	CO4	CO3	Ruchi
15%	66.60 ± 0.64	83.30 ± 0.84	73.30 ± 0.47	86.60 ± 0.78
20%	50.00 ± 0.43	53.30 ± 0.49	60.00 ± 0.52	46.60 ± 0.31
25%	33.30 ± 0.38	36.60 ± 0.21	30.00 ± 0.29	40.00 ± 0.25
30%	23.30 ± 0.26	20.00 ± 0.18	23.30 ± 0.22	20.00 ± 0.16
SEd	0.36771			
CD (p<0.05)	0.74903			
CD (p<0.01)	1.00699			

Values are mean ± SD of three samples in each group

Figure-1

Germination



Experiment 2

Growth attribute

Plant height

Under stress the plants showed morphological changes and it was evidently seen in our research. The variety under stress showed reduced leaf size and drying of the tips. Water stress decreased plant height in all varities through the magnitude of the effect of stress. A maximum plant height of (44.0cms) in ruchi to a minimum of (20.67cms) was observed in control plants (Table 2 and Figure 2).

At moisture stress, the maximum height decreased to7cms in local variety and to 4cms in CO4 due to stress. A maximum plant height of 38.67cm (Ruchi) and minimum of 15cms in CO3 was recorded. This study results finds out that plant height was declined by drought stress. The decrease in shoot lenght may be either due to decrease in cell elongation resulting from by choi *et al* 2000 and singh *et al* (2000). The reduction of shoot lenght may protect the loss of water by mechanisms of migirate drought stress.

Koehler *et al*., (1982) also observed that stalk elongation as expressed by plant height in drought stressed plants was less than 80% of the plants in well-watered plots. A strong and positive relationship between stalk elongation and water content was reported by Shih and Gascho (1980). In the case of sugarcane, the reduction in shoot height indicated the reduction in final sink size. The cell expansion rather than the cell division appeared to be sensitive to water stress, which might be the cause for reduction in plant height.

Plant weight

At moisture stress, in general all the varities showed reduction in weight and variety CO4 showed the maximum reduction 28.8% (Table3 and Fig 3). A maximum plant weight was recorded in local variety (2.18gms) and minimum of (1.24gms) in ruchi was recorded in normal watering. In drought stressed plants the weight varied between (1.1 to 2.1gms).

Reduced dry weight of plant in bean was reported by Enam *et al* (2010). The growth of plants was effected by the drought stress, where drought had highly significant effect on shoot fresh and dry weight, root fresh and dry weight and significant effect on the shoot-root ratio of these weights in Marigold (Riaz *et al* 2013). Reduced shoot, dry weight under drought in coriander was also reported by Rajabi and Fetri (2013)

Plate-3

Coriander leaves under normal water

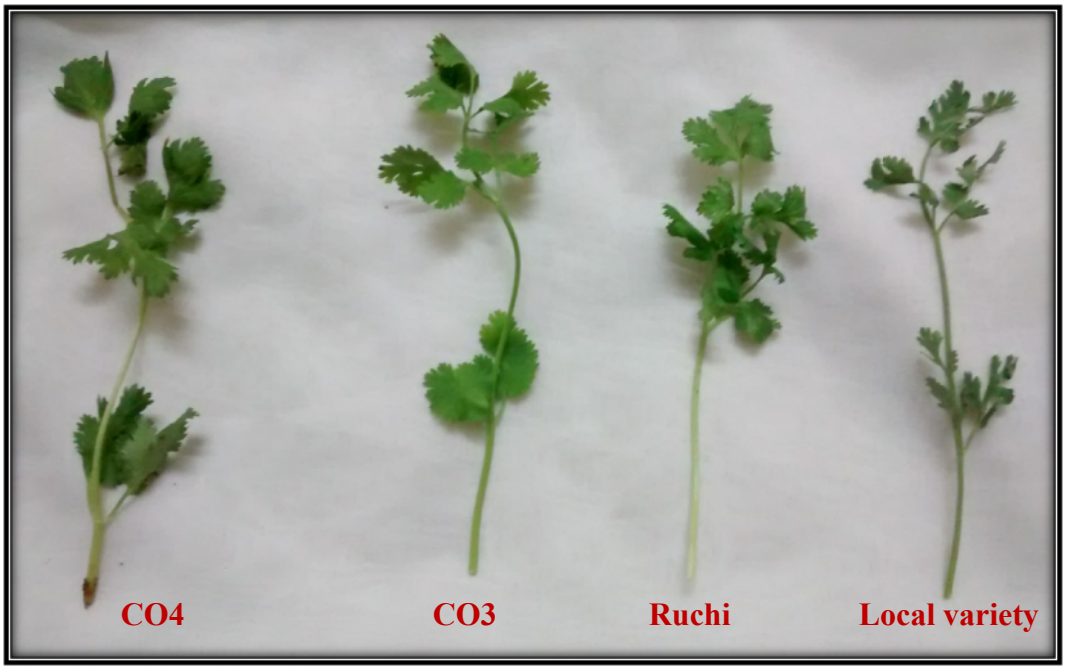


Plate-3a

Coriander leaves under stress water

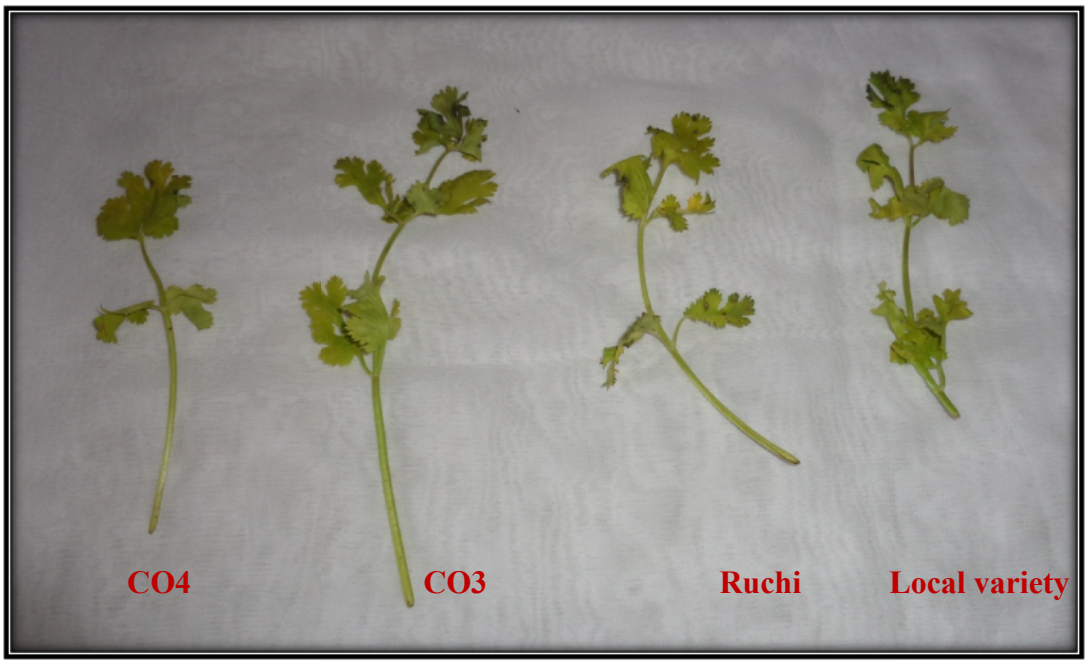


Table-2

Plant height (cms) of four coriander varieties during stress

Plant Variety	Control	Treatment
I. Local Variety	26.17 ± 2.75	19.00 ± 2.00
II. CO4	32.07 ± 2.10	28.00 ± 2.00
III. CO3	20.67 ± 3.06	15.00 ± 2.00
IV. Ruchi	44.00 ± 3.00	38.67 ± 1.53
SEd CD ($p<0.05$) Cd ($p<0.01$)	1.92837 4.08802 5.63257	

Values are mean ± SD of three samples in each group

Figure-2

Plant height (cms) of four coriander varieties during stress

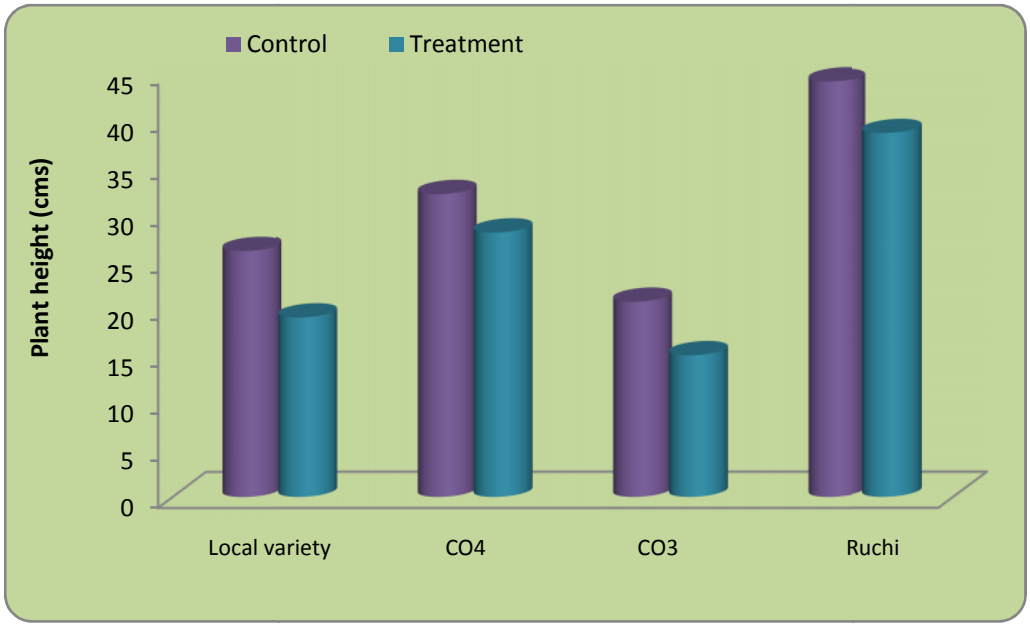


Table-3

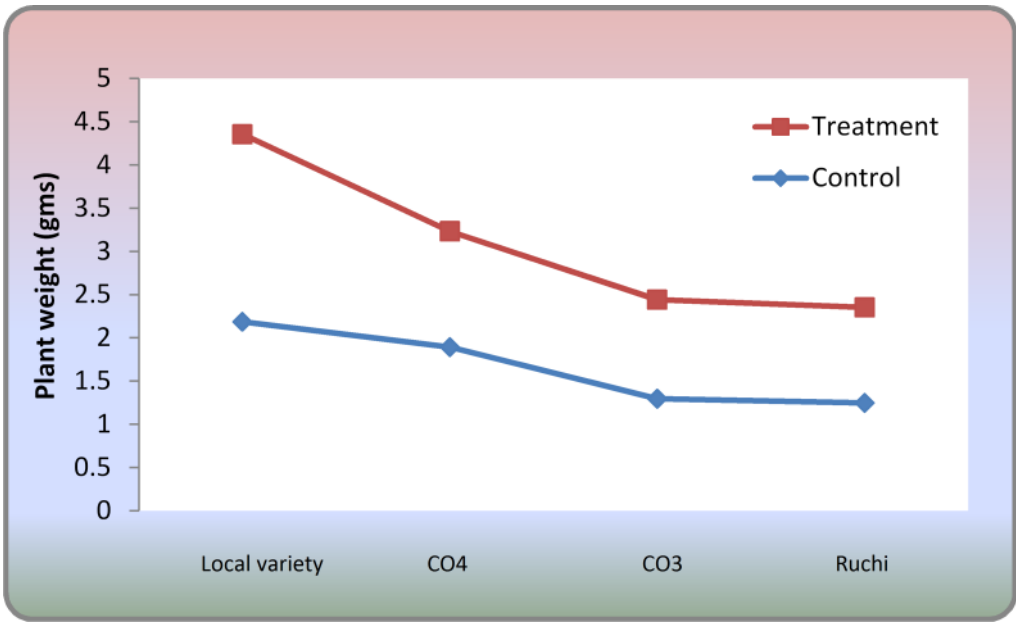
Plant weight (gms) of four coriander varieties during stress

Plant Variety	Control	Treatment
I. Local Variety	2.187 ± 0.002	2.167 ± 0.003
II. CO4	1.893 ± 0.004	1.346 ± 0.002
III. CO3	1.296 ± 0.003	1.145 ± 0.003
IV. Ruchi	1.248 ± 0.003	1.105 ± 0.004
SEd CD ($p<0.05$) Cd ($p<0.01$)	0.00230 0.00487 0.00671	

Values are mean ± SD of three samples in each group

Figure-3

Plant weight (gms) of four coriander varieties during stress



Proline accumulation

After stress, ruchi, accumulated maximum proline of 83.75 ug fr wt^{-1} while CO3 recorded a minimum of 29.24 ug g fr wt. At stress, all the varieties showed about 20% more increase in proline and ruchi recorded the highest of 63% (Table 4 and Figure 4).

In response to water stress, proline accumulation generally occurs in the cytosol where it plays significant role in cytoplasmic osmotic adjustment (Anjum *et al*., 2011). Proline accumulation enabled water stressed plants to maintain low water potentials, by reducing the water potential, it allowed additional water to the taken up, thus counteracting the influence of drought stress on plant tissues (Kumar *et al*., 2003).

The accumulation of proline in leaves under water stress has been widely reported (Aspinal and Paleg, 1981). In the present investigation, coriander varieties were found to accumulate proline at significantly higher concentrations. At stress, all varieties showed 100% increase in proline, and local variety recorded a maximum of 36.51 ug g fr wt^{-1} as compared to CO4 which has accumulated minimum proline 46.04 ug g fr wt^{-1}. Proline is commonly accumulated by stressed plants and may be responsible to serve as nitrogen buffer and energy for recovery after alleviation of stress. Moore and Maretzki (1975) reported increase concentrations of several aminoacids especially proline in coriander. Similar findings were already reported in coriander (Rao and Asokan, 1978, Singh and Singh, 1986). Hanson *et al*. 1977 also reported that free proline increased more in a drought sensitive than in a drought resistant cultivar of barley.

The higher magnitude of proline may help plants to tolerate dehydration by maintaining cell turgidity as reported earlier by Sivakumar *et al*. (1998). Proline also protected and stabilized the protein, DNA membranes (Matysik *et al*., 2002).

Table-4

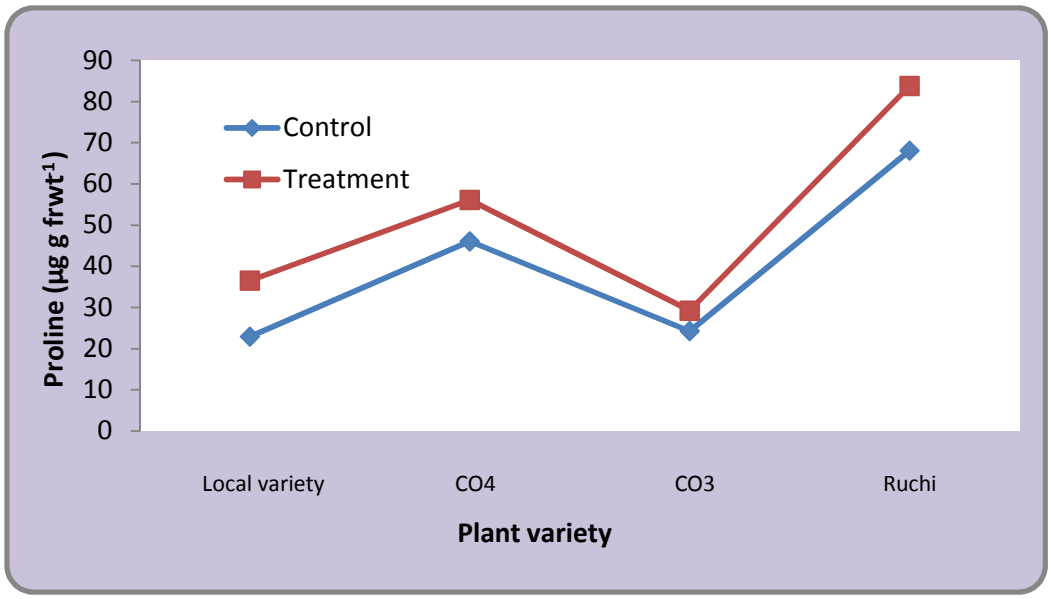
Proline accumulation (µg g frwt-^{1}) in coriander varieties during stress

Plant Variety	Control	Treatment
I. Local Variety	22.94 ± 2.05	36.51± 2.78
II. CO4	46.04 ± 5.27	56.10± 2.58
III. CO3	24.29 ± 1.54	29.24 ± 2.57
IV. Ruchi	68.05 ± 3.08	83.75 ± 2.76
SEd CD ($p<0.05$) Cd ($p<0.01$)	2.45588 5.20631 7.17338	

Values are mean ± SD of three samples in each group

Figure-4

Proline accumulation (µg g frwt-^{1}) in coriander varieties during stress



Glycine betaine

Glycine betaine accumulation under stress varied between 1.85 in local to 3.14ug in ruchi. There was two fold increase in glycinebetaine under stress in ruchi and CO4 and comparatively ruchi showed maximum accumulation (50% increase). The treatment at differences were statistically significant (Table 5 and figure 5).

Glycine betaine, is considered as one important, predominant and most effective osmoprotectant (Burnet *et al*., 1995). Which increase the tolerance of plants to drought (Raza *et al*., 2012b).

The accumulation of glycine betaine has been reported under water stress conditions and has been considered to possess important physiological role in the osmoregulation of the cytoplasmic cell components, protein protection and membrane stabilization about 78% increase in glycine betaine was observed under stress. Glycine betaine accumulation at local variety 1.85 µg dr wt^{-1} to 2.24 µg dr wt^{-1} (CO4) in control plants, while in stressed plants it varied from 1.91 µg dr wt^{-1} (CO3) to 3.14 µg dr wt^{-1} (ruchi). A positive correlation of glycine betaine with yield was inferred from the experiment. It was also reported in other crops that glycine betaine accumulation positively correlated with increased tolerance to dehydrations under drought (Saneoka *et al*., 1995). Glycine betaine was known to stabilize the protein structure of PS-II complex and prevent membrane damage from the stress by membrane stabilization (Rudolph *et al*., 1986, Yang *et al*., 1996).

Pigments

Pigments viz., total chlorophyll, chlorophyll a and b were quantified during stress in control and stressed plants. The total chlorophyll content varied between 2.63 to 1.76 in control and 2.25 to 1.17 in stress (Table 6 and Figure 6). Variety CO4 showed the highest chlorophyll content even under stress and decrease in chlorophyll content was informly in all varieties but local variety showed maximum reduction (42%) the chlorophyll a and b ratio was also influenced by stress and were reduced (Table 7 and 8, Figure 7 and 8). Similarly there was reduction in total carotenes and xanthophylls. carotenoids under stress was maximum in ruchi 0.885 showing its tolerance.

Table-5

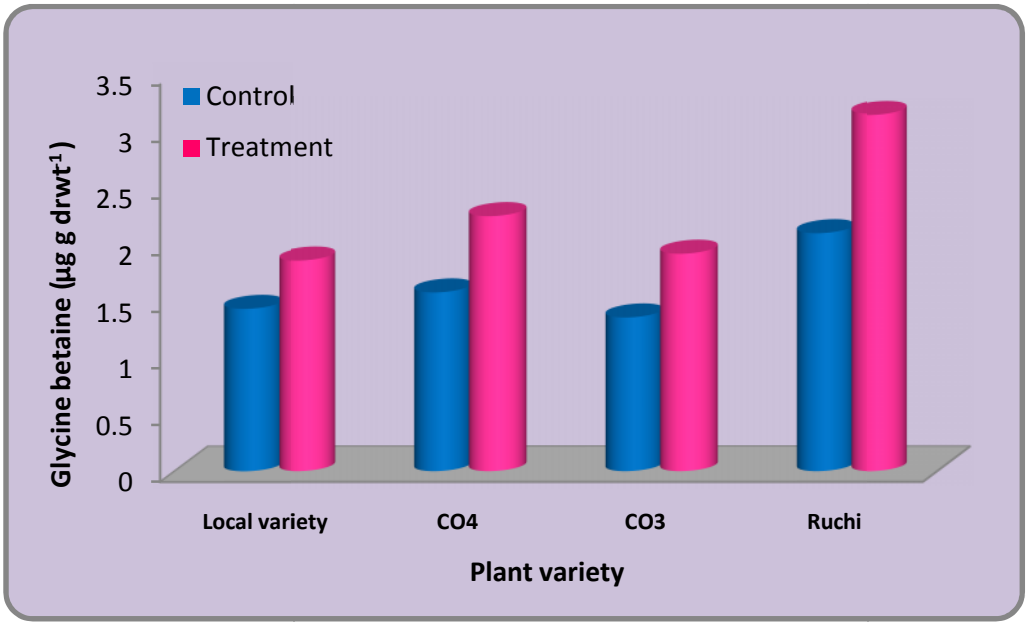
Glycine betaine accumulation (µ g drwt-^{1}) in four coriander varieties during stress

Plant Variety	Control	Treatment
I. Local Variety	1.43 ± 0.01	1.85 ± 0.00
II. CO4	1.57 ± 0.01	2.24 ± 0.05
III. CO3	1.35 ± 0.02	1.91 ± 0.35
IV. Ruchi	2.09 ± 0.32	3.14 ± 0.20
SEd CD (p<0.05) Cd (p<0.01)	0.14870 0.31524 0.43435	

Values are mean ± SD of three samples in each group

Figure-5

Glycine betaine accumulation (µ g drwt-^{1}) in four coriander varieties during stress



Carotenes

Chlorophyll and carotenoid pigments showed significant quantiitative varation due to moisture stress. The total chlorophylls were 1.07mg/g to 2.25mg/g in treatment and 1.86mg/g to 2.26mg/g in treatment reduction due to stress treatment. CO4 variety maintained a higher pigment concentration under stress.

The tolerant varieties generally possessed high pigment concentration which was contrary in case of susceptible types. Hence pigments and chlorophyll stability index may be used for screening drought tolerance. Reduced chlorophyll stability index under drought has been reported in maize by Meenakumari *et al*., (2004). Decreases in chlorophyll/ carotenoid ratio could be used as an indicator of tolerance status of the plants under stress (Daymi *et al*., 2005) and it was also documented that the effect of water stress on pigments differ depending on plant species, severity and duration of drought (Munne and Penuelas, 2004).

The total carotenoids ranged from a maximum of 0.909 in ruchi to a minimum of 0.366 in local variety in normal plants. Under stress the carotenoids levels varied from 0.885 to 0.234mg/g and variety ruchi maintained higher levels of carotenoids (Table 9 and 10, Figure 9 and 10). Similarly xanthophylls were also decreased under stress CO4 recorded minimum xanthophylls (0.109 mg/g $frwt^{-1}$) and ruchi maximum (0.809 mg/g $frwt^{-1}$) (Table 11 and Figure 11). The variety, treatment and the interactions differed significantly.

Retardation in the content of photosynthesis pigments in response to water stress was attached to the structural deformation of plastids forming the thylakoids (Maslenkova and Toncheva, 1997., Zhang *et al* 2006). Photosynthesis pigment degradation exposed to water stress in rice (Basu *et al*., 2010) wheat (Salekjalali *et al*., 2011) and increased Setaria(Ajithkumar and Panneerselvam, 2013) and this degradates is due to oxidation of photosynthesis pigments used damaged chloroplasts. According to our data stressed plants the chlorophyll pigments were reduced.

Paramasivam et *al*. (2009) have reported photosynthetic pigments and carotenoids show multifarious roles in drought tolerance including light harvesting and production from oxidative damage caused by drought. Mafakheri *et al*., (2010) showed that the chlorophyll a was more sensitive to drought stress than chlorophyll b. Shamsi *et al*. (2010) reported the effects of drought stress on chlorophyll content in bread wheat cultivars under field conditions. The results showed that with an increase in the intensity of drought stress on wheat cultivars, there was a decrease in total chlorophyll content.

Balakumar *et al*. (1988) reported reduction in chlorophyll propotional to carotenoids in cotton and sorghum to moisture stress. Shamsi *et al*. (2010) studied the effects of drought stress on chlorophyll content in bread wheat cultivars under field conditions. The results showed that with an increase in the intensity of drought stress on wheat cultivars, there was a decrease in total chlorophyll content. Bhosale *et al.* (2011) reported that the amount of chlorophyll content was found to be decreased due to increase in water stress.

Drought stress responses of wheat genotypes with respect to nutritional quality (Gupta *et al*., 2012) reported that drought resistant cultivars, as compared to sensitive genotypes, maintain higher chlorophyll content under drought stress condition. Similarly Semsettin *et al*. (2012) reported that the effect of chl a, chl b, total carotenes and showed reduction under stress treatments. Kanokporn *et al*. (2012) reported that under water deficit condition, pigmented rice seedlings showed the retardant in growth and photosynthetic pigments.

Studies carried out by Siosemardeh *et al*., (2010) to assess drought sensitive varieties in chickpea to limited water supply showed similar patterns of decreased chlorophyll a, b, a/b concentrations. Reduced chlorophyll content was later reported in ginger by Shinde *et al*. (2011) and in coriander Aliabadi farahani *et al*. (2008).

Antioxidant

Superoxide dismutase (SOD)

During strees, SOD activity increased in all the varieties. The activity ranged from 6.09 in local variety to 10.70 in CO3 in non stress plant as compared to 11.57 (Ruchi) to 15.74 (CO3) in stressed plant. All stressed varieties showed 40% increase in SOD activity invariable, the treatment, variety and their intraction were statistically significant (Table 12 and Figure 12).

Table-6

Effect of moisture stress on total chlorophyll content (mg/g frwt^{-1})

Plant Variety	Control	Treatment
I. Local Variety	1.86 ± 0.03	1.07 ± 0.03
II. CO4	2.63 ± 0.03	2.25 ± 0.03
III. CO3	1.76 ± 0.03	1.66 ± 0.04
IV. Ruchi	2.08 ± 0.05	1.17 ± 0.03
SEd CD ($p<0.05$) Cd ($p<0.01$)	0.02609 0.05530 0.07620	

Values are mean ± SD of three samples in each group

Figure-6

Effect of moisture stress on total chlorophyll content (mg/g frwt-$^{1)}$

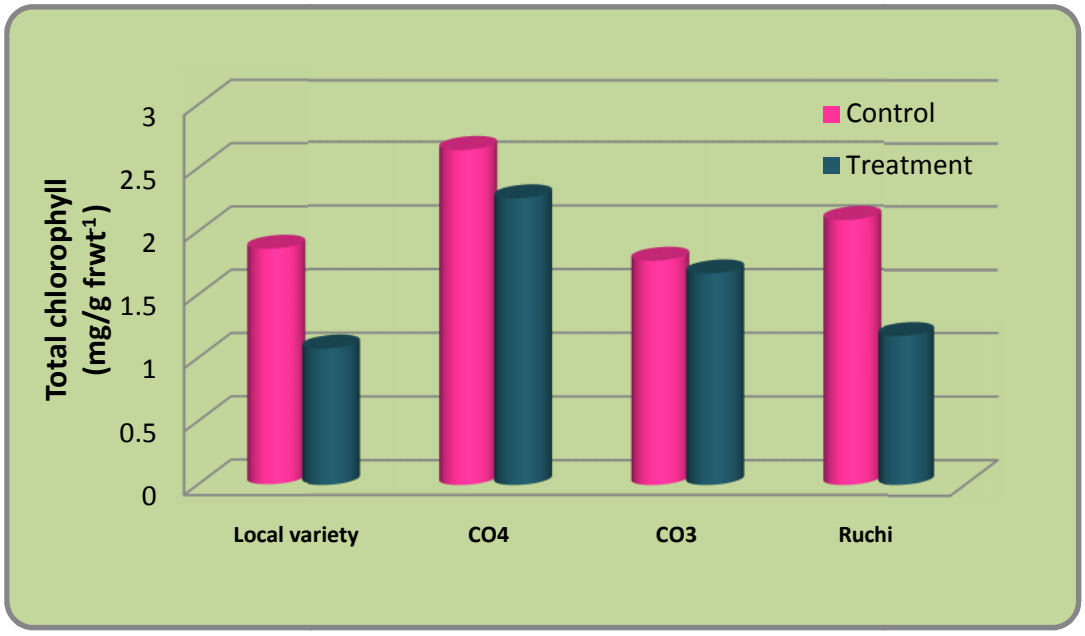


Table-7

Effect of moisture stress on chlorophyll a content (mg/g frwt^{-1})

Plant Variety	Control	Treatment
I. Local Variety	0.285 ± 0.049	0.245 ± 0.003
II. CO4	0.865 ± 0.004	0.476 ± 0.003
III. CO3	0.676 ± 0.003	0.454 ± 0.003
IV. Ruchi	0.524 ± 0.003	0.613 ± 0.003
SEd CD ($p<0.05$) Cd ($p<0.01$)	0.01419 0.03007 0.04143	

Values are mean ± SD of three samples in each group

Figure-7

Effect of moisture stress on chlorophyll a content (mg/g frwt^{-1})

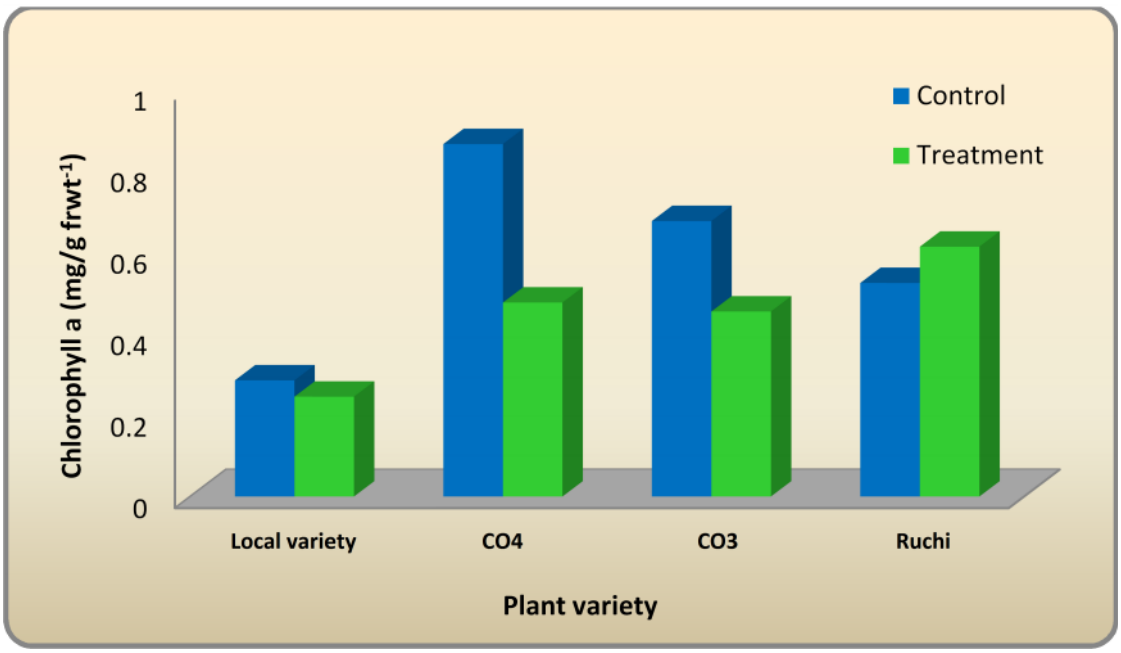


Table-8

Effect of moisture stress on chlorophyll b content (mg/g frwt-$^{1)}$

Plant Variety	Control	Treatment
I. Local Variety	0.835 ± 0.004	0.504 ± 0.004
II. CO4	0.629 ± 0.003	0.594 ± 0.003
III. CO3	0.308 ± 0.003	0.274 ± 0.004
IV. Ruchi	0.478 ± 0.004	0.443 ± 0.003
SEd CD (p<0.05) Cd (p<0.01	0.00265 0.00562 0.00774	

Values are mean ± SD of three samples in each group

Figure - 8

Effect of moisture stress on chlorophyll b content (mg/g frwt^{-1})

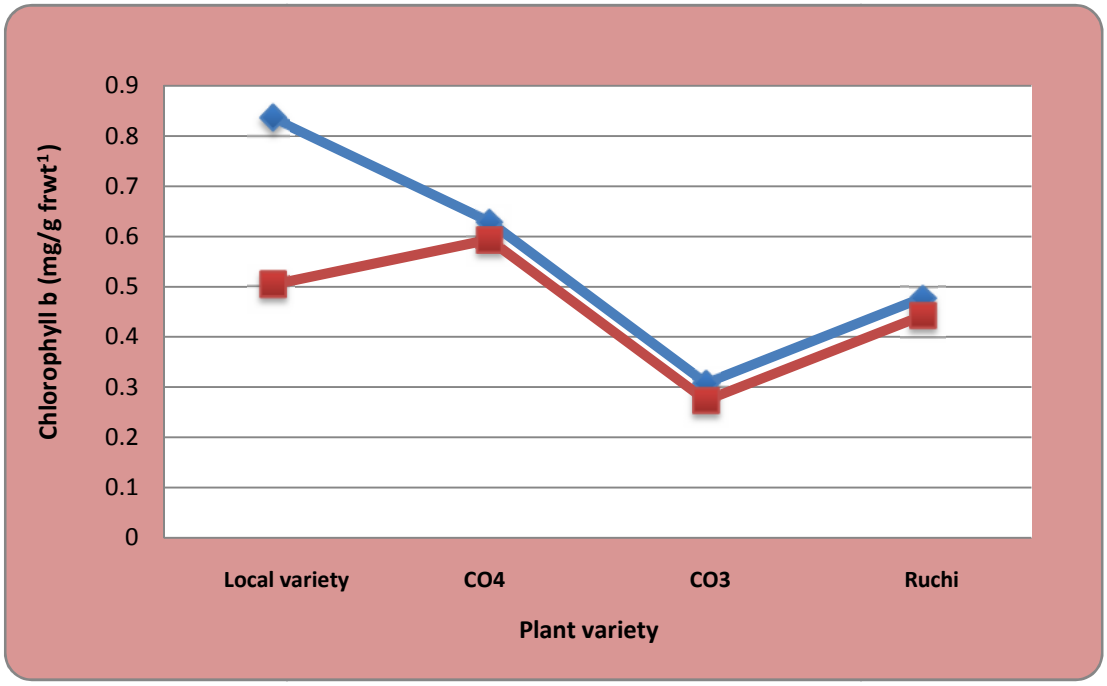


Table-9

Effect of moisture stress on total carotenoids content (mg/g frwt$^{-1)}$

Plant Variety	Control	Treatment
I. Local Variety	0.366 ± 0.003	0.234 ± 0.003
II. CO4	0.644 ± 0.004	0.574 ± 0.003
III. CO3	0.425 ± 0.004	0.385 ± 0.003
IV. Ruchi	0.909 ± 0.002	0.885 ± 0.004
SEd CD ($p<0.05$) Cd ($p<0.01$)	0.00253 0.00537 0.00740	

Values are mean ± SD of three samples in each group

Figure - 9

Effect of moisture stress on total carotenoids content (mg/g frwt^{-1})

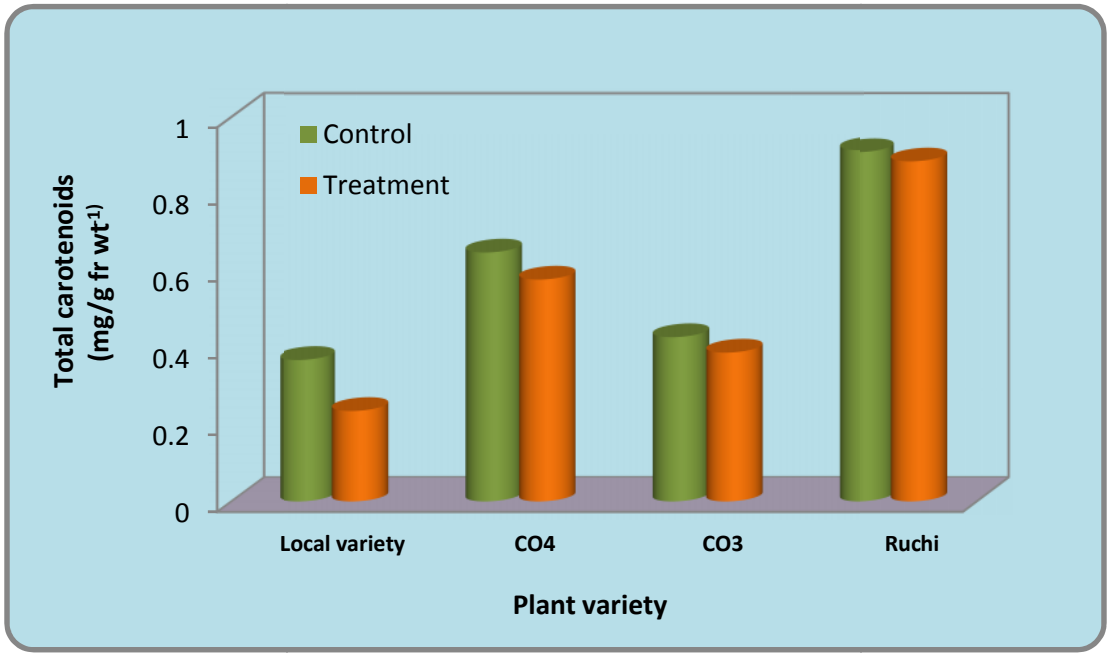


Table-10

Effect of moisture stress on total carotenes content (mg/g frwt^{-1})

Plant Variety	Control	Treatment
I. Local Variety	0.695 ± 0.003	0.544 ± 0.003
II. CO4	0.764 ± 0.003	0.684 ± 0.002
III. CO3	0.386 ± 0.003	0.298 ± 0.003
IV. Ruchi	0.725 ± 0.003	0.605 ± 0.004
SEd CD ($p<0.05$) Cd ($p<0.01$)	0.00231 0.00490 0.00675	

Values are mean ± SD of three samples in each group

Figure-10

Effect of moisture stress on total carotenes content (mg/g frwt^{-1})

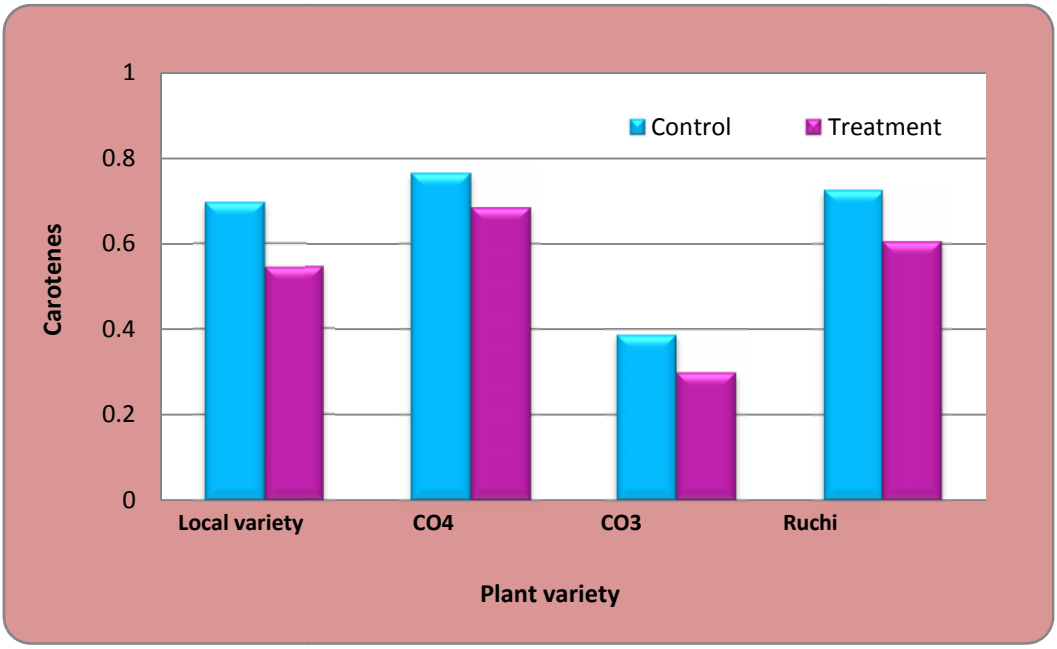


Table-11

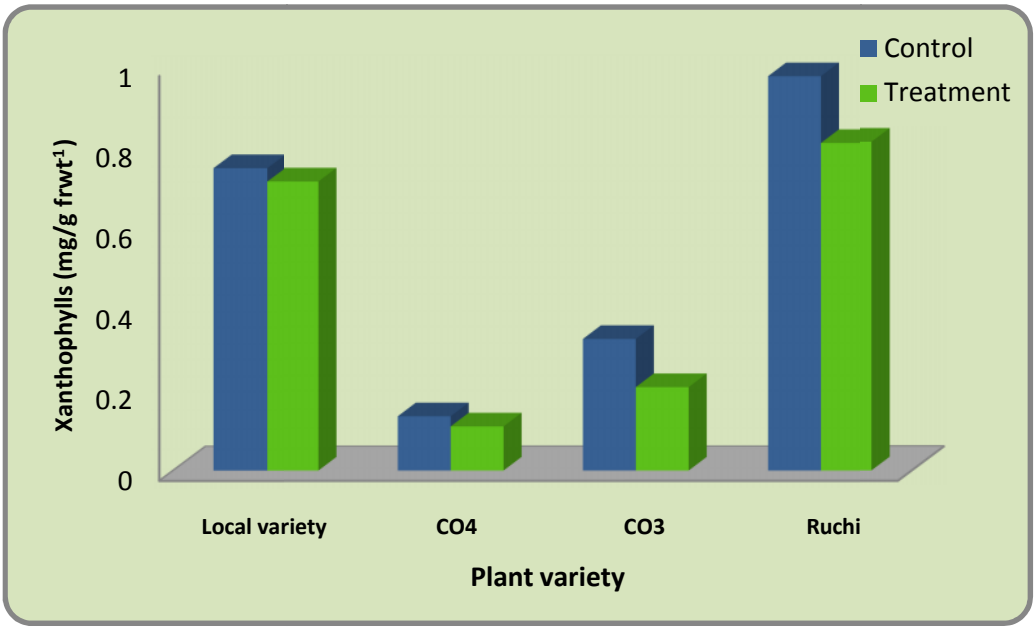
Effect of moisture stress on xanthophylls content (mg/g frwt^{-1})

Plant Variety	Control	Treatment
I. Local Variety	0.747 ± 0.003	0.714 ± 0.004
II. CO4	0.134 ± 0.003	0.109 ± 0.003
III. CO3	0.325 ± 0.004	0.206 ± 0.002
IV. Ruchi	0.974 ± 0.003	0.809 ± 0.004
SEd CD (p<0.05) Cd (p<0.01)	0.00253 0.00537 0.00740	

Values are mean ± SD of three samples in each group

Figure-11

Effect of moisture stress on xanthophylls content (mg/g frwt^{-1})



Antioxidants enzymes such as SOD, POX, APX and CAT were reported to increase under environmental stress. As a confirmation, in the present work, we also observed enhanced actived of SOD and POX under drought stress. They are the important components of protective system scavenge O_2 and H_2O_2 respectively. Other authors have pointed out that drought and saline stress increase SOD activity (Dhindsa and Matowe, 1981., Dhindsa *et al*., 1982., Gaspar and Dhindsa 1981., Kalirand Poljakoff Mayber, 1981).

Among the antioxidants, SOD is essential components of defense mechanism in plants under environmental adversity (Bowler *et al*., 1992). Water stress did not influence SOD in sorghum (Zhang and Kirkham, 1996) and wheat (Sairam *et al*., 1998., Sairam and Srivastava, 2001).

POD

At maximum stress, a peroxidase activity increased appreciably recording about 120% increase. In normal plants peroxidase activity ranged from 4.16 to 6.77, while it varied from 9.8 to 12.10 under stress. A maximum peroxidase of 12.10 was recorded in CO3 (Table 13 and Figure 13).

POD enzyme scavenges a part of ROS and protects the photosynthetic machinery (Sheoran and Garg, 1979; Egert and Tevini, 2002; Rios- Conzalez *et al*., 2002; Candan and Tarhan, 2003). Under water deficiency conditions O_2 and H_2O_2 increased since the stomata get closed (Larson, 1988). As a result, a significant increase in the content of antioxidant enzymes, like peroxidase was noticed (Larson, 1988., Egert and Tevini. 2002, Yordanov *et al*., 2000). Peroxidases catalyze oxidation of various substrates in the cell (Miller *et al*., 1990) and significant roles of peroxidises have been suggested in plants.

Yang-Li *et al*., (1995) also reported increased POX activity in response to drought treatment in resistant genotypes of sugarcane. Increased POX activity was reported in maize (Zhang *et al*., 1995), grasses (Fu and Huang, 2001), cucumis sativus (Tekchandani and Guruprasad, 1998) and wheat (Li and Liang 1988) in response to drought, decreased in sunflower and sorghum seedlings (Zhang and Kirkham, 1996) and no changes in wheat (Fangmeiner *et al*., 1994) in response to water stress. Similar results of increased oxidative enzymes under drought stress in tolerant wheat genotypes have been reported (Sairam *et al*., 1998). Chugh and Kaur (2013) the role of oxidative stress management was evaluated in two

maize genotypes to drought stress at reproductive stage. POX activity was also influenced in both genotypes with increase in drought stress.

Esen and Ozgur (2012) determined the antioxidant system responses of a xerophytic plant *Gypshophila aucheri* to drought and reported increased POX activity

The effects of soil water shortages on the activity of antioxidant enzymes in Barley showed significant increases in POX activity in both moderate and severe stress treatments (Salekjalali and Haddad, 2012). Similar finding was also reported in soybean (Mohamed and Akladious, 2014).

Proteins

The total proteins were estimated and the quantity was 11.27, 13.18, 18.54, and 27.93 mg-^{100gm} in local, CO4, CO3 and ruche respectively in normal plants. Under stress protein were reduced in quantity in all the varities and a maximum protein was content seen in ruchi (Table 14 and Figure 14).

The leaf protein profiles through native PAGE revealed the occurrence of 2-8 band during stress with molecular weight ranging from 205 KDA to 14.3 KDA. During stress call the four varieties showed similar banding patterns and no additional bands were observed in drought stressed samples, but the intensity of the bands varied showing more concentration under control samples.

Protein is the performer of life activity, the change of plant morphology corresponds with the change of relative proteins (Singh *et al*., 1993). There is response of protein composition to environmental factor especially water stress. Severe drought stressed wheat seed, protein bands through SDS-PAGE revealed no additional bands, but the drought stressed sample bands were chromatin, due to high protein concentration (Parchin and Shaban 2014). Similar findings were reported by (Javid *et al* 2004, Iqbal *et al* 2005).

Table-12

Effect of moisture stress on superoxide dismutase (SOD) activity (Unt mg^{-1})

Plant Variety	Control	Treatment
I. Local Variety	6.09 ± 0.03	13.74 ± 0.02
II. CO3	9.37 ± 0.03	12.57 ± 0.03
III. CO4	10.70 ± 0.03	15.74 ± 0.03
IV. Ruchi	8.24 ± 0.04	11.57 ± 0.03
SEd CD ($p<0.05$) CD ($p<0.01$)	0.02236 0.04740 0.06531	

Values are mean ± SD of three samples in each group

Figure-12

Effect of moisture stress on superoxide dismutase (SOD) activity (Unt mg^{-1})

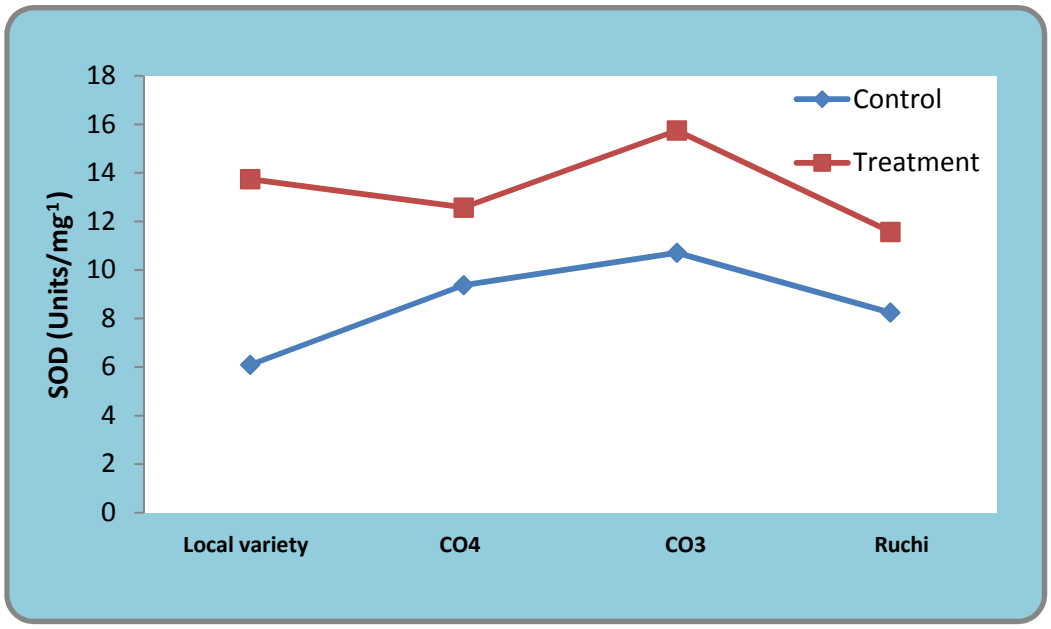


Table-13

Effect of moisture stress on peroxidase activity (Unt mg^{-1})

Plant Variety	Control	Treatment
I. Local Variety	5.06 ± 0.04	10.67 ± 0.02
II. CO4	6.77 ± 0.04	12.10 ± 0.03
III. CO3	5.44 ± 0.35	12.02 ± 0.03
IV. Ruchi	4.16 ± 0.03	9.28 ± 0.03
SEd CD ($p<0.05$) CD ($p<0.01$)	0.10277 0.21786 0.30017	

Values are mean ± SD of three samples in each group

Figure-13

Effect of moisture stress on peroxidase activity (Unt mg^{-1})

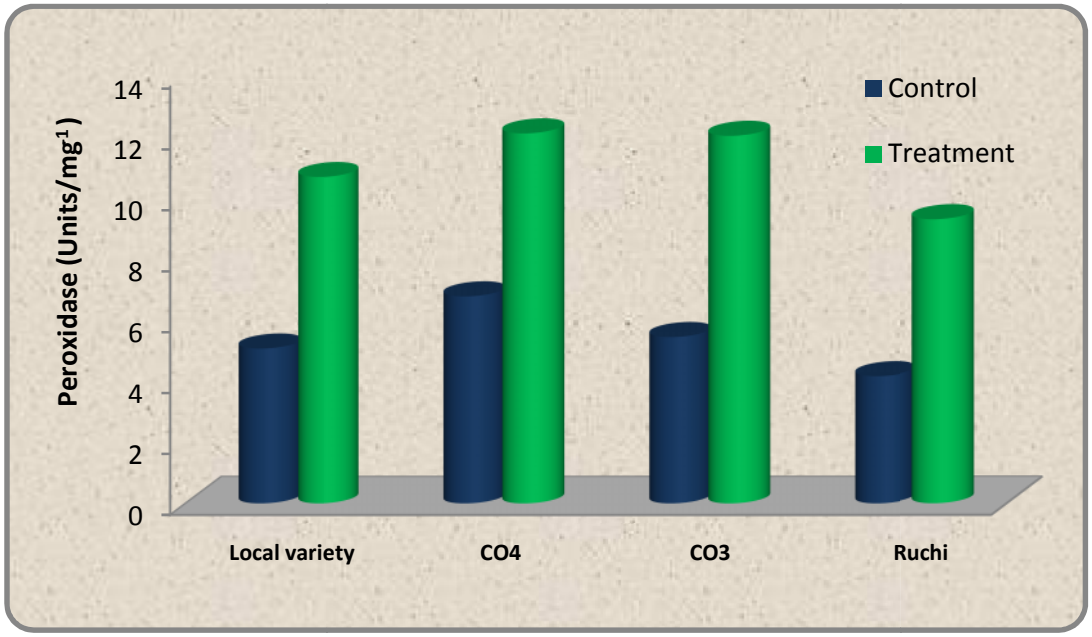


Table - 14

Estimation of protein content ($mg^{-100}gm$) under stress

Plant Variety	Control	Treatment
I. Local Variety	11.72 ± 0.18	7.27 ± 0.18
II. CO4	13.18 ± 0.33	9.15 ± 0.38
III. CO3	18.54 ± 0.24	11.36 ± 0.72
IV. Ruchi	27.93 ± 0.32	11.06 ± 0.30
SEd	0.30116	
CD ($p<0.05$)	0.63843	
Cd ($p<0.01$)	0.87964	

Values are mean ± SD of three samples in each group

Figure-14

Estimation of protein content ($mg^{-100}gm$) under stress

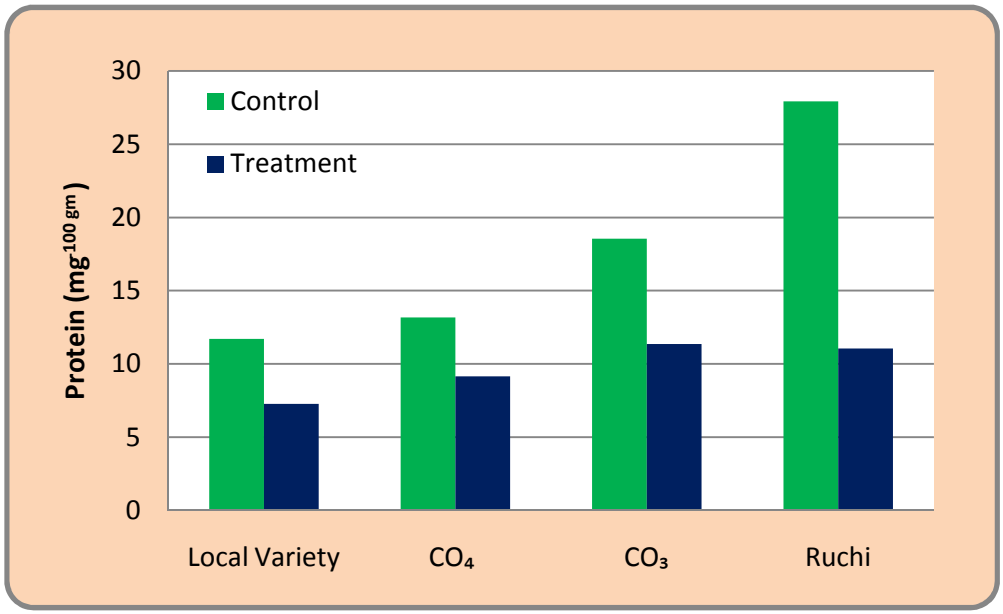
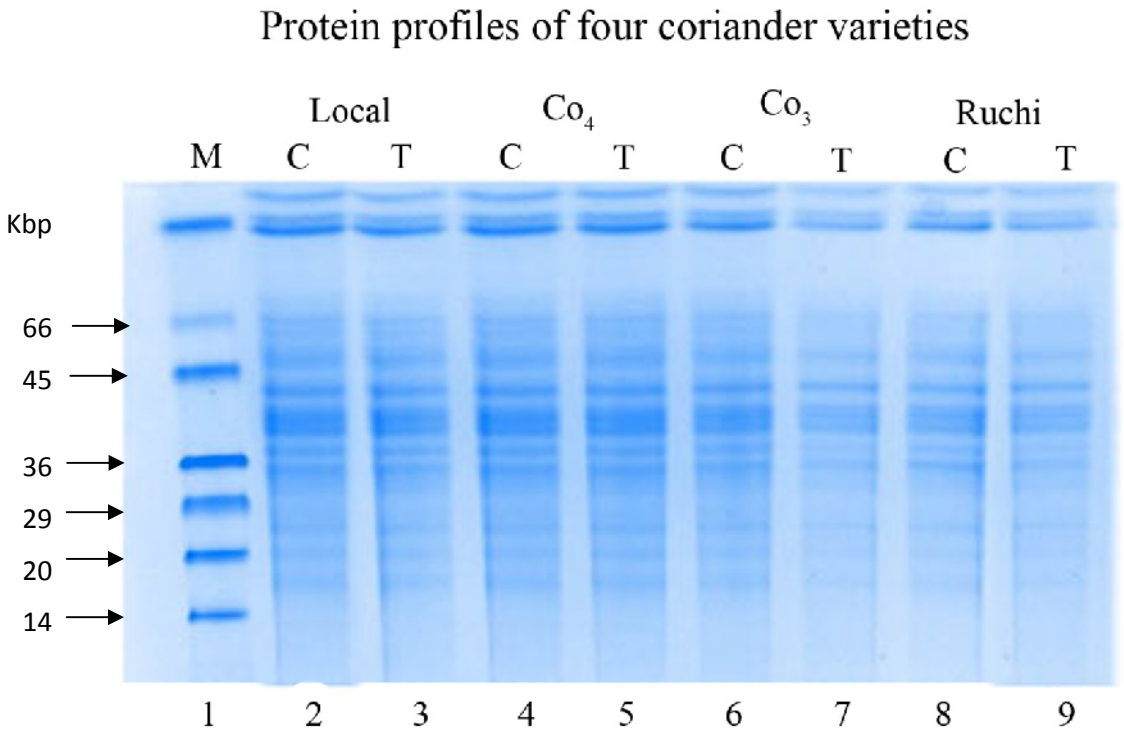


Plate - 4


Protein profiles of four coriander varieties
Local
Co4
Co3
Ruchi
M
C
T
C
T
C
T
C
T
Kbp
66
45
36
29
20
14
1
2
3
4
5
6
7
8
9

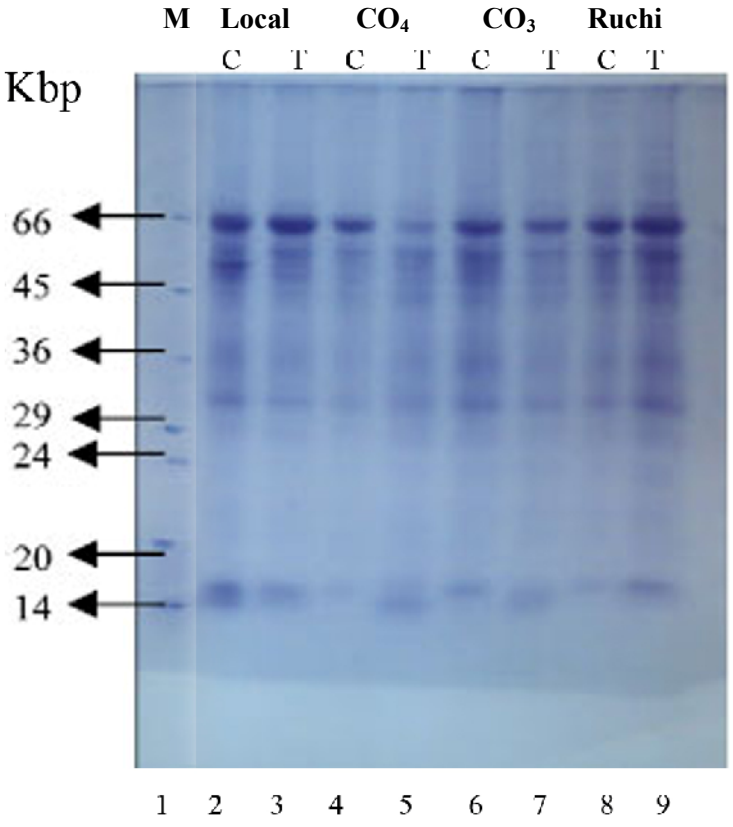
Isoenzyme pattern of SOD

Owing to the higher activity of SOD, this enzyme was further analysed for its isoforms through PAGE. Under stress, all the four varieties showed 7 isoforms bands and no similar banding pattern was also observed in control samples. Comparing the stressed and control lane, the intensity of bands with higher in stressed treatment lanes froming the higher activity of SOD once again.

Five major bands with SOD activity was detected in coriander leaf sample ranging between 14KDA to 66KDA Kbp molecular weights. The four varieties studies showed similar isoenzyme banding patterns, but the intensity of the treatment lanes was higher compared to control. No additional bands were detected under drought stress.

Plate-5

Isoenzyme banding pattern of SOD in four coriander varieties



CHAPTER – V

SUMMARY AND CONCLUSION

A pot culture experiment was conducted at Avinashilingam University, Coimbatore, TamilNadu, during 2014-2015, utilizing four coriander varieties (*Coriandrum sativum* L.) four varieties of coriander (*Coriandrum sativum* L.) such as Local, Ruchi, CO4, CO3 to study effect of drought stress.

The impacts of drought stress on various morphological characters such as plant height, plant weight were recorded during growth in normal and stressed plants. Drought treatment caused an average reduction of about 34% in plant height while tolerant types such as local variety and CO4 showed less reduction under drought.

The plant growth and productivity was assessed through photosynthetic rate which directly involved photosynthetic pigments which was considerably affected due to drought. Reductions in photosynthetic pigments were prominent under stress. Drought has altered the concentration of chlorophylls and carotenoid pigments and xanthophylls.

In response to drought, certain varieties have shown a high osmotic potential due to increased accumulation of osmolytes such as proline and glycine betaine. Proteins have revealed reduction in concentration suggestion damage due to drought stress. Protein banding pattern also revealed no additional band formation, supporting the fact that in coriander under drought additional proteins not formed. Increased activities of antioxidant enzymes (SOD and POD) were observed and this helps the plant to cope up with the ROS damage. Antioxidant enzyme SOD was analyzed for it isoforms through PAGE which showed no additional bands under stress, but the intensity of bands varied which proved higher concentration of enzyme under stress.

From this study it is inferred that when coriander undergoes drought stress there is a reduction in germination, followed by growth and at biochemical level the plant system undergoes damage to its pigment system due to ROS scavenging. To maintain the osmotic potential in stressed plants accumulation of osmolytes (proline and glycine betaine) occurred and to counteract the ROS damage increased levels of antioxidant were observed which further supported the plant to cope with drought stress.

REFERENCES

Abdollah. N., Nahid Niari Khamssi, Ali Mostafaie and Hosein Mirzaee, 2010. Effect of progressive water deficit stress on proline accumulation and protein profiles of leaves in chickpea, **African Journal of Biotechnology, 9(42):** 7033-7036.

Abedi, T., and Hassan Pakniyat, 2010. Antioxidant enzymes changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.), **Czech J. Genet. Plant Breed., 46(1):** 27-34.S

Ahmad, P, 2010, growth and antioxidants response in mustard (*Brassica junica* L.) plants subjected to coined effect of glycine betaine and salinity, **Arch. Agron. Soil. Sci., 56(5):** 578-588.

Ahmad, P. Jaleel, C. A. And Sharma, S, 2010. Antioxidant defense system, lipid peroxidation, proline melabolizcy enzymes and biochemical activity of two genotypes of *Morus alba* L. Subjected to Nacl stress, **Russ. J. Plant Physiol., 57(4):** 509-517.

Alcazar, R., Altabella, T. Mano, F. Bottowtti, R. Reymond, M. Konez, C, 2010. Polyamines, molecules with regulatory function in plant abiotic stress tolerance, **Planta, 231(1):** 237-249.

Amita, S. M.D, Shamim, K. N. Singh, 2013. Genotypic variation in root anatomy, starch accumulation, and protein induction in upland rice (*Oryza sativa*) varieties under water stress, **Agric res 2(1):** 24-30.

Anonymous 2013. Indian horticulture database, **National horticulture board,** 6.

Ardic, M., A.H. Sekmen, I. Turkan, S.Tokur and F.ozdemir, 2009. The effects of boron toxicity on root antioxidant systems of two chickpea (Cicer arietinum L.) cultivars, **Plant Soil., 314:** 99-108.

Arisi, A., Cornic, G., Jovanin, L. and Foyer, C.H. 1998. Over expression of superoxide dismutase in transformed modified the regulation of photosynthesis at low CO_2 partial pressures or following exposure to pro-oxidant herbicide methyl viologen. **Plant Physiol., 117**: 565-574.

Arora, A., R.K. Sairam and G.C. Srivastava, 2002. Oxidative stress and antioxidative systems in plants, **Curr. Sci., 82:** 1227-1238.

Arumingtyas, E.L., Evika Sandi Savitri, Runik Dyah Purwoningrahayu, 2013. Protein Profiles and dehydrin accumulation in some soybean varieties (*Glycine max* L. Merr) in drought stress conditions, **American Journal of Plant Sciences, 4:** 134-141.

Asokan, S. 1978. Studies on free proline association to drought resistance in sugarcane. **Sugar J., 40**: 23-24.

Aspinall, D. and Paleg, L.G. 1991. Proline accumulation. Physiological aspects In: The physiology and biochemistry of drought-resistance in plants, Paleg, L.G. and Aspinall, D. (eds.), Academic Press, New York, 206-240.

Azaz, K., S.A. Mallick, Moni Gupta, Sachin Gupta, and A.k. Mondal, 2012. Drought stress responses of wheat genotypes with respect to nutritional quality, **Indian J Agric Biochem., 25(2):** 94-99.

Bajji, M., Lutts, S. and Kinet, S.M. 2000. Physiological changes after exposure to and recovery from PEG induced water deficit in callus cultures issued from durum wheat (*Triticum durum* Desp.) cultivars differing in drought resistance. *J. Plant Physiol*., **156**: 75-83.

Balakumar, T., Selvaraj, A. and Damayanthi, N. 1988. Growth and physiological responses of cotton and sorghum to flooding stress. *Intern. Congress of Plant Physiology,* **54**, New Delhi.

Bates, L.S. Waldeen, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, **39**: 205- 208.

Beauchamp, C. and Fridovich, J. 1971. Superoxide Dismutase: Improved assay and an assay applicable to acrylamide gels. *Ann. Biochem*., **444**: 274-287.

Bhosale, K.S., and B.P. Shinde, 2011. Influence of arbuscular mycorrhizal fungi on proline and chlorophyll content in *Zingiber officinale* rose grown under water stress, **Indian Journal of fundamental and Applied Life Sciences, 1(3):** 172-176.

Blum, A. and Ebercon, A. 1976. Genotypic responses in sorghum to drought stress III. Free proline accumulation and drought resistance**. Crop Sci., 16:** 428-431.

Bohnert, H. J., D.E. Nelson and R.G. Jenson, 1995. Adaptations to environmental stresses, **Plant cell, 7:** 1099-1111.

Bowler, C., Van Montague. and Inze, M.D. 1992. Superoxide dismutase and stress tolerance. **Ann. Rev. Plant Physiol.** *Plant Mol. Biol*., **43**:83-116.

Boyer, J.S, 1982. Plant productivity and environment, **Sci, 218:** 443-448.

Burk, J. and Oliver, T. 1992. Differential sensitivity of pea SODs. *Plant Physiol*., **100**: 1595-1598.

Burnet, M.P. J. Lafontaine and A.D. Hanson, 1995. Assay, purification and partial characterization of choline monoxygenase from spinach, **Plant physiol., 168:** 581-588.

Candan, N. and Tarhan, L. 2003. Relationship among chlorophyll carotenoid content, antioxidant enzyme activities and lipid peroxidation levels by Mg^{2+} deficiency in the *Mentha pulegium* leaves. *Plant Physiol. Biochem*., **41**: 35-40.

Cattivelli, L. Rizzia, F. Badeck, F.W. Mazzucotelli, E. Mastrangelo, A.M. Francia, E. Mare,C. Tondelli, A. Stanca, A.M, 2008. Drought tolerance improvement in crop plants: An investigated view from breeding to genomics, **Field crop res., 105:** 1-4.

Chanan, I.A. and Ali, N 1988. Is proline involved in the regulation of stomatal movement. *International congress of Plant Physiology*., **98:**131.

Chen, W. P., Li, P. H. Chen, T.H.H, 2000. Glycine betaine increase chilling tolerance and reduces chilling- induced lipid peroxidation in *Zea mays* L. **Plant cell environ**.,**23:** 609-618.

Chopra, R., and D.S. Selote, 2007. Acclimation to drought stress generates oxidative stress tolerance in drought- resistant than susceptible wheat cultivars under field conditions, **Environ. Ex Bot., 60:** 276-283.

Chutipaijit, S., Suriyan Cha-Um and Kanokporn Sompornpailin, 2012. An evaluation of water deficit tolerance screening in pigmented indica rice genotypes, **Pak. J.Bot., 44(1):** 65-72.

Corso, G.M., Brinholi, O., Machado, S.R. and Factori, V. 1981. Comparative anatomical study of leaves of sugarcane varieties submitted to water deficit treatments. **Brasil acuc., 98**: 146 –152.

Dawar, S., Vani, T. and Singhal, G.S. 1998. Stimulation of antioxidant enzymes and lipid peoxidation by UV-B irradiation in thylakoid membranes of wheat. **Biol. Plant., 41**: 65-73.

Daymi, C., Pedro, R., Angeles, M.M., Jose, M.D.A., Arturo, T. and Juan, J.A. 2005. High temperature effects on photosynthesis activity of two tomato cultivars with different heat susceptibility. **J. Plant Physiol., 162**: 281-289.

De Ronde, J.A., Cress, W.A., Kruger, G.H.J, Strasser, R.J. and Van Staden, J. 2004. Photosynthetic response of transgenic soybean plants containing an *Arabidopsis* P5CR gene, during heat and drought stress. **J. Plant. Physiol., 161**:1211-1224.

Dhindsa, R.S. and Matowe, W.1981. Drought tolerance in two mosses Correlated with enzymatic defence against lipid peroxidation. **J. Exp. Bot., 32**: 79-91.

Dhindsa, R.S., Plumb-Dhindsa, P.L. and Reid, D.M. 1982. Leaf senescence and lipid peroxidation: effects of some phytohormones and scavengers of free radicals and singlet oxygen. **Physiol. Planta., 56**: 453-457.

Douglas, T.J., and Paleg, L.G. 1981. Lipid composition of *zea mays* seedlings and water stress induced changes. **J.Exp. Bot., 32**: 499-508.

Egert, M. and Tevini, M.2002. Influence on some physiological parameters symptomatic for oxidative stress in leaves of chives (*Allium schoenoprasum*). **Env. Exp. Bot., 48**: 43-49.

Esen, A. H. S., Rengin Ozgur, Baris Uzilday, Zehra Ozgecan Tanyolac, Ahmet Dinc, 2012. The response of the xerophytic plant *Gypsophila aucheri* to salt and drought stresses: the role of the antioxidant defence system, **Tubitak, 36:** 697-706.

Ewase, A.E.D.S.S., Samira Omran, Soad El-Sherif, and Nagat Tawfik, 2013. Effect of salinity stress on coriander (*Coriandrum Sativum*) seeds germination and plant growth, **Egypt. Acad. J. Biolog. Sci., 4(1):** 1-7.

Fu, J. and Huang, B. 2001. Involvement of antioxidants and lipid peroxidation in the adaptations of two cool-season grasses to localized drought stress. *Env. Exp. Bot*., **45**: 105-144.

Gal, G. Anwer, M.M, Meena, S.S, Mehta, R.S. Maeria, S.P, 2010. Advances in production technology of coriander, **National research center on seed spices Ajmer Raj,** 1-5.

Gaspar, C.L. and Dhindsa, R.S. 1981. Correlation between germination and activities of SOD and POX under salinity in rice (*Oryza sativa* L.). *Plant Physiol*., **67**: 19.

Ghamarnia, H., Saba Daichin, 2013. Effect of different water stress regimes on different coriander (*Coriander Sativum* L.) Parameters in a semi-arid climate, **International Journal of Agronomy and Plant Production, 4(4):** 822-832.

Girija, C., Smith, B.N. and Swamy, P.M. 2002. Interactive effect of sodium chloride and calcium chloride on the accumulation of proline and glycine betaine in peanuts (*Arachis hypogea* L.). **Environ. Exp. Bot., 47**: 1-10.

Gosavi, S.P., P.K.Lokhande, U.S. Dalvi, P.N. Harer and R.M. Naik, 2014. Biochemical assessment of chickpea genotypes for drought tolerance in relation to osmolytes and limiting amino acids in protein, **Indian. J. Agric. Biochem., 27(1):** 45-51.

Grattan, S.R. and Grieve, C.M. 1985. Betaine status in wheat in relation to nitrogen stress and to transient salinity stress. **Plant Soil., 85**: 1-10.

Hadas, A., 1977. A simple laboratory approach to test and estimate seed germination performance under field conditions, **Agronomy journal., 69:** 582-588.

Hanson, A.D. 1980. Interpreting the metabolic responses of plants to water stress. **Hort. Science., 15**: 623-629.

Hanson, A.D., Nelsen,C.E. and Everson, E.H. 1977. Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. **Crop Sci., 17:** 720-726.

Hemaprabha,G. and Sree Rangasamy, S.R. 2001. Genetic similarity among five species of *saccharum* based on isozyme and RAPD markers. **Indian J. Genet., 67**: 341-347.

Hsu, S.Y., Hsu, Y.T. and Kao, C.H. 2003. The effect of polyethylene glycol on proline accumulation in rice leaves. **Biolgia Plantarum, 46**: 73-78.

Indira, P. and Kabeerathuma, S. 1986. Effect of water stress during different phases of tuberisation in sweet potato. Summary of papers of the workshop on "Impact of drought on plantation crops". 47.

Iqbal, N., M.Y. Ashraf and M.Ashraf, 2005. Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage, **Int. J. Environ. Sci. Tech., 2(2):** 155-160.

Iqbal, N., M.Y. Ashraf and M.Ashraf, 2005. Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage, **Int. J. Environ. Sci. Tech., 2(2):** 155-160.

Iwai, S., Kawashima, N., Matsuyama, S. 1979. Effects of water stress on proline catabolism in tobacco leaves. **Phytochemistry, 18**: 1155-1157.

Khan, M.I. and Garg, O.P. 1981. Effect of water stress on nucleic acid and protein metabolism of maize at different stages of growth and development. **Indian J. Plant Physiol. 24:** 150-157.

Koehler, P.H., Moore, P.H., Jones, C.A., Cruiz, A.D. and Maretzki, A. 1982. Response of drip-irrigated sugarcane to drought stress. *Agron. J*., **74**: 906-911.

Kramer, P.J. and Boyer, J.S, 1995. Water relations of plants and soils., **Academic press, sandiego, 19:** 56-63.

Kumar, I.P.A., R. Panneerselvam, 2013. Osmolyte acculumation, Photosynthetic Pigment and growth of *Setaria italic* (L.) P. *Beauv.* Under drought stress,**Asian Pacific Journal of Reproduction, 2(3):**220-224.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, **227**: 680-685.

Larson, R.A. 1988. The antioxidants of higher plants. *Photochemistry,* **24**: 969-978.

Lowery, O.H., N.J. Roserbourgh, A.L. Farr and R.F. Randall, 1951. Protein measurement with Folin-phenol reagent, **J. Biol. Chem., 193 (7):** 257-275.

Ludlow, M. M. Muchow, R. C. A, 1990. A critical evaluation of traits for improving crop yields in water- limited environments, **Advances in agronomy 43:** 107-153.

Mafakheri, A., A. Siosemardeh, B.Bahramnejad, P.C .Struik, Y. Sohrabi, 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars, **Australian Journal of Crop Science, 4(8):** 580-585.

Mafakheri, A., A. Siosemardeh, B.Bahramnejad, P.C .Struik, Y. Sohrabi, 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars, **Australian Journal of Crop Science, 4(8):** 580-585.

Malik, C.P. and Singh, M.B. 1980. In: Plant enzymology and histoenzymology. Kalyani Publishers, New Delhi. 53.

Mansour, M.M, 1998. Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against Nacl stress, **Plant Physiol Biochem., 35:** 767-772.

Matysik,J., Alia, G., Bhalu, B. and Mohanty, P.2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci*., **82**: 525-531.

Mayer, A., Mayber, A.P, 1989. The germination of seeds, **Pergamon Press., 67:** 44-50.

Meena, M.L. Kumar, V. Kumar, S. Yadav, Y.C. Kumar, A, 2010. Genetic variability, heritability, genetic advance, correlation coefficient and path analysis in coriander, **Indian journal of horticulture, 67:** 242-246.

Meenakumari, S.D., Vimala, Y. and Pawan, A. 2004.Physiological parameters governing drought tolerance in maize. *Indian J. Plant Physiol*., **9**: 203-207.

Miller, R., Kelly, J.T. and Mujer, V.C. 1990. Anodic peroxide isoenzymes and polyphenol oxidase activity from cucumber fruit tissue and substrate specificity. *Phytochemistry*, **29**: 705-709.

Misra, N. and A.K. Gupta, 2006. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in *Catharanthus roseus* seedlings, **J. Plant Physiol., 163:** 8-11.

Mohammadkhani, N. Heidari, R, 2008. Effect of drought stress on soluble proteins in two maize varieties, **Turk J Biol., 32:** 23-30.

Mohanty, A. Kathuria, H. Ferjani, A. Sakamoto, A. Mohanty, P. Murata, N. Tyagi, F.K, 2002. Transgenics of an edible indica rice variety *Pusa basmati* I. Harbouring the cod A gene on highly tolerant to salt stress, **Theor. Appl. Genet. 106:** 55-57.

Moller, I. M. Jensen, P.E. Hansson, A, 2007. Oxidative modifications to cellular components in plants, **Annual review of plant biology, 58:** 459-481.

Momin, A.H., S. Sawapnil, Acharya and Amit V. Gajjar, 2012. *Coriandrum Sativum*-Review of advances in phytopharmacology, **International Journal Pharmaceutical Sciences and Research, 3(5):** 1233-1239.

Moradpour, K., A. Najaphy, S. Mansoorifar, A. Mostafaie, 2014. Evaluation of leaf protein pattern in wheat genotypes under drought stress, **Journal of Advanced Biological and Biomedical Research, 2(3)**840-846.

Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. **Ann. Rev. Plant Physiol., 35**: 299-319.

Mukherjee, I. 1980. Genotypic differences in potassium responses and proline accumulation in maize during wilting. **Plant Cell Physiol., 21**: 197-200.

Munne, B.S. and Penuelas, J. 2004. Drought induced oxidative stress in strawberry tree (*Arbutus unedo* L.) growing in Mediterranean field conditions. **Plant Sci., 116**: 1105-1110.

Munns, R., 2005. Genes and salt tolerance: bringing them together. **New physol., 167:** 645-663.

Munns, R., Brady, C.J., and Barlow, E.W.R. 1979. Solute accumulation in the apex and leaves of wheat during water stress**. Aust. J. Plant Physiol., 6:** 379-389.

Neto, N. B. M, Saturnino, S,M, Bomfim, D.C. Custodio, C.C, 2004. Water stress induced by mannitol and sodium chloride in soyabean cultivars, **Brazilian biology and technology 47:** 521-529.

Noctor, G. And C. Foyer, 1998. Ascorbate and glutathione: keeping active oxygen under control, **Annu. Rev. Plant physiol., 49:** 249-279.

Okcu, G., Kaya, M.D. Atak, M, 2005. Effects of salt and drought stresses on germination and seedling growth of pea *(Pisum sativum* L.), **Turkian J. Agric., 29:** 237-242.

Oliveria, M.J. Norsworthy, J.K, 2006. Pitted morning-glory(*Ipomoe lalacunosa*) germination and emergence as affected by environmental factors and seedling depth. **Weed science 54:** 910-916.

Paramasivam, m., Jaleel, C. Abdul Wahid, Muhammad Farooq, Hameed Jasim Al-Juburi, Ramamurthy Somasundaram and Rajaram Panneerselvam, 2009. Drought stress in plants: a review on morphological characteristics and pigments composition, **International Journal of Agricultural and Biology, 11(1):** 100-105.

Parameshwara, G., Paleg, L.G., Aspinall, D. and Jones, G.P.1988. Solute accumulation in *alfalfa* in response to environmental stresses.**International congress of plant physiology, 128.**

Parchin, R.A., and Morad Shaban, 2014. Protein profiles and seeds storage proteins changes in wheat genotypes under control and drought stress conditions, **Scientia Agriculturae, 1(1):** 6-8.

Phutela, A., Jain, V., Dhawan, K. and Naina, H.S. 2002. Water stress induced changes in growth characteristics and proline content in seedlings of *B. junceae* cultivars differing in drought tolerance. *Cruciferae Newsletter*, **24**: 49-50.

Pugnaire, E.L., Endolz L.S. and Pardos, J. 1994. In. Constraints by water stress on plant growth. Pessarakli, M. (ed). **Handbook of plant crop stress.** Marcel Dekker, New York, 247-259.

Rajabi, M., and Moslem Fetri, 2013. Effect of drought and salinity stress on germination and seedling growth of coriander (*Coriandrum Sativum*), **International Journal of Farming and Allied Sciences, 2 (16):** 510-513.

Raza, M.A.S., M.F. Saleem, M.Y. Ashraf, A.Ali and H.N. Asghar, 2012b. Glycinebetaine applied under drought improved the physiological efficiency of wheat (*Triticum aestivum* L.) plant, **Soil environ., 31(1):** 67-71.

Raza, M.A.S., Muhammad Farrukh Saleem, Moazzam Jamil and Imran Haider Khan, 2014. Impact of Foliar Applied Glycinebetaine on Growth and Physiology of Wheat (*Triticum aestivum L.*) Under Drought Conditions,**Pak.J. Agri. Sci., 51(2):**327-334.

Raza,M.A.S., M.F.Saleem, G.M.Shah, I.H.Khan and A.Raza, 2014. Exogenous application of glycinebetaine and potassium for improving water relations and grain yield of wheat under drought, **Journal of Soil Science and Plant Nutrition, 14(2):**348-364.

Reddy, P.C. and Vajranabhaiah, S.N. 1993. Drought induced lipid peroxidation: defense mechanism in upland rice (*Oryza sativa* L.) seeds during germination. *Adv. Plant Sci*., **6**: 229-236.

Rhodes, D. and Samaras, Y. 1994. Genetic control of osmoregulation in plants. In: Cellular and molecular physiology of cell volume regulation. Strange K. Boca: CRC Press.347-361.

Riaz, A., Adnan Younis, Asif Riaz Taj, Asmat Karim, Usman Tariq, Shoaib Munir and Sitwat Riaz, 2013.Effect of drought stress on growth and flowering of marigold (*Tagetes erecta* L.), **Pak. J. Bot., 45(1):**123-131.

Rios-Gonzalez,K., Eradi,L. and Lips,S.H.2002. The activity of antioxidant enzymes in sources. **Plant Sci.. 162**: 923-930.

Roberts D.G., Patricia, G.W. and Daniel, M.R. 1984. Glycine betaine content of halophytes: Improved analysis by liquid chromatography and interpretations of results. **Physiol. Plant**. **61**: 195-202.

Rudolph, A.S., Crowe, H. and Crowe, L.M. 1986. Effects of three stabilizing agents: proline, betaine and trehalose on membrane phospholipids. **Arch. Biochem. Biophys., 245**: 134-143.

Sairam, R.K. and Srivastava, G.C. 2001. Water stress tolerance of wheat (*Triticum aestivum* L) variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. **J. Agron. Crop Sci., 6**: 229-236.

Sairam, R.K., Deshmukh, P.S and Saxena, D.C. 1998. Role of antioxidant systems in wheat genotypes tolerance to water stress. **Biol. Plant., 41**: 387-394.

Salekjalali, M., Raheem Haddad. And Behboud Jafari, 2012. Effects of soil water shortages on the activity of antioxidant enzymes and the contents of chlorophylls and proteins in barley, **American-Eurasian J. Agric. And Environ. SCI., 12(1):** 57-63.

Saneoka, H., Nagasaka, C., Hahn, D.T., Yang, W.J., Premachandra, G.S., Joly, R.J. and Rhodes, D. 1995. Salt tolerance of glycine-betaine deficient and containing maize lines. *Plant Physiol*., **107**: 631-638.

Saneoka, H., Nagasaka, C., Hahn, D.T., Yang, W.J., Premachandra, G.S., Joly, R.J. and Rhodes, D. 1995. Salt tolerance of glycine-betaine deficient and containing maize lines. *Plant Physiol*., **107**: 631-638.

Sarwar,M.K.S., Ihsan Ullah, Mehboob-UR-Rahman, M.Yasin Ashraf and Yusuf Zafar, 2006. Glycinebetaine accumulation and its relation to yield and yield components in cotton genotypes grown under water deficit condition, **Pak. J. Bot., 38(5):** 1449-1456.

Saxena, S.N., R.Kakani, R.Saxena and M.M.Anwer, 2010. Effect of water stress on seed quality of coriander (*Coriandrum Sativum* L.), **Journal of Spices and Aromatic Crops, 19(1 & 2):** 53-56.

Shahbaz, M., Y.Masood, S.Perveen, M.Ashraf, 2011. Is foliar- applied gilcinebetaine effective in mitigating the adverse effects of drought stress on wheat (*Triticum aestivum* L.), **Journal of Applied Botany and Food Quality, 84:** 192-199.

Shahzad, M. K, 2006. Glycine betaine accumulation and its relation to yield and yield components in cotton genotypes grown under with deficit conditions, **Pak. J. Bot., 38(5):** 1449-1459.

Shamsi, K., 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars, **Journal of Animal and Plant Sciences, 8(3):**1051-1060.

Sharma, A.D., Gurmeen Rakhra, Supreet Kaur and Hardeep Kaur, 2013. Expression of Bioling-Stable Peroxidase (PRX) isoenzymes under combined effect of drought and heat in different tissues of *Triticum aestivum*, **Journal of Agricultural Technology, 9(4):** 901-913.

Sharma, P. Dubey, R.S, 2005. Lead toxicity in plants, **Braz. J. Plant Physiol**., **17:** 35-52.

Sheoran, I.S. and Garg, O.P. 1979. Quantitative and qualitative changes in peroxidases during germination of mung bean under salt stress. **Physiol Plant., 46**: 147-150.

Shih, S.F. and Gascho, G.J. 1980. Relationship among stalk elongation, leaf area and dry biomass of sugarcane. *Agron. J*., **72**: 309-313.

Singh, A.K., Chaudhry, R.K. Shaema, R.P.R, 1993. Effect of inoculation and fertilizer level on yield, nutrients uptake and economics of summer pulses, **Ind. J. Potassium. Res., 9:**176-178.

Singh, T.N., Aspinall, D. and Paleg, L.G. 1972. Proline accumulation and varietal adaptability to drought in barley: a potential metabolic measure of drought resistance. **Nature, 236:** 188-190.

Singh, T.N., Aspinall, D., Paleg, L.G. and Boggers, S.F. 1973. Stress metabolism II. Changes in proline concentration in excised plant tissues. **Aust. J. Biol. Sci., 26**: 57-63.

Sivakumar, V., Ramachandran, K., Ravichandran, V. and Vanangamudi, M. 1998. Effect of drought hardening on proline content of seedlings. **Ann.Plant Physiol., 12**: 82-84.

Stewart, C.R. 1981. In: Physiology and Biochemistry of drought resistance. Paleg, L.G. and Aspinall, D (eds.), Sydney**, Academic press**. 243 – 258.

Sumithra, K. and Reddy, A.R. 2004. Changes in proline metabolism of cowpea seedlings under water deficits. **J. Plant Biol., 31**: 201-204.

Tan, B.H. and Halloran, G.M. 1982. Variation and correlations of proline accumulation in spring wheat cultivars. **Crop sci., 22**: 459-463.

Tekechandani, S. and Guruprasad, K.N. 1998. Response of C*alamagrastis arundinaceae* and *C. epigeios* to short and long term water stress. **Biol. Plant., 42**: 129-131.

Terzi, R., and Asim Kadioglu, 2006. Drought stress to tolerance and the antioxidant enzymes system in *Ctenanthe setose*, **Acta Biologica Cracoviensia Series Botanica, 48 (2):** 89-96.

Tomader, E, Christophe, B., Gandonon, F., Hayat, E., Jawal, A., Motramed, I. and Nadia, S.S. 2006. Growth, proline and ion accumulation in sugarcane callus cultivars

under drought-induced osmotic stress and its subsequent relief. **African Journal of Biotechnology, 5:** 1488-1493.

Tsugane, K. Kobayashi, K. Niwa, Y. Ohba, Y. Wada, K. Kobayashi, H, 1999. A recessive Arabidopsis mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification, **Plant cell, 11:** 1195-1206.

Tulley, R.E., Hanson, A.D. and Nelson, C.E. 1979. Proline accumulation in water stressed barley leaves in relation to translocation and the nitrogen budget. **Plant Physiol., 63**: 518-523.

Unyayar, S., Yuksel Keles and Elif Unal, 2004. Proline and ABA levels in two sunflower genotypes subjected to water stress, **Scientific and technical research council of turkey, 52:** 301-308.

Wahid., A. Rasul, E. Rao, A.R, 1997. Germination responses of sensitive and tolerant sugarcane lines to sodium chloride, **Seed Sci. And Technol., 25:**467-470.

Wang, E., 2010. Over accumulation of glycine betaine enchances tolerance to drought and heat stress in wheat leaves in the protection of photosynthetic pigment, **Photosynthetica, 48(1):** 121-126.

Weybrew, J.A. 1957. Estimation of the plastid pigments of tobacco. **Tobacco Science**, **144:** 18-22.

Yang, G, Rhodes, P. and Jolly, R.J. 1996. Effects of high temperature on membrane stability and chlorophyll flourescenec in glycine-betaine deficit and gylcine-betaine containing maize lines. **Aus. J. Plant Physiol., 23**: 437-443.

Yang-Li, Tao, Li-Yang, Rui, Yang, L.T. and Li, Y.R. 1995. Studies on the effect of late irrigation on sugarcane yield, sucrose content and some physiological and biochemical characters of different sugarcane cultivars under drought conditions. **Acta Agronomica sinica., 21**: 76-82.

Yordanov, I., Velikova, V. and Tsonev, T. 2000. Plant responses to drought, acclimation and stress tolerance. **Photosynthetica., 38**: 171-186.

Zhang, J. and Kirkham, M.B. 1996. Antioxidant responses to drought in sunflower and sorghum seedlings. **New Phytol., 132**: 361-373.

Zhang, J., Cui, S., Li, J. and Kirkham, M.B. 1995. Protoplasmic factors, antioxidant response and chilling resistance in maize. **Plant Physiol. Biochem., 33**: 567-575.

Zhaolong, W. and Bingru, H. 2004. Physiological recovery of Kentuky bluegrass from simultaneous drought and leaf stress. **Crop Sci., 44**: 1729-1736.