V. DISCUSSION

The main objective of the present investigation is to evaluate the effect of drought stress and its amelioration by exogenous application of salicylic acid (SA) and jasmonic acid (JA) in *Allium cepa* L. Var. *Aggregatum* (shallot). The results of parameters analyze are discussed hereunder.

1. Growth parameters

1.1. Root length

Root length of *Allium cepa* decreased under drought stress as compared to control. Drought stress decreased the root length in *Albizzia* seedlings (Sundaravalli et al., 2005), *Eucalyptus microtheca* seedlings (Li et al., 2000) and *Populus* species (Yin et al., 2005). Similar results were observed under drought stress in sorghum (Maryam Naikan et al., 2013), *Glycine max* (Heba and Samai, 2014) and spring wheat genotypes (Irshad et al., 2014). Drought stress affects both elongation and expansion the rate of cell expansion, and ultimately, cell size and consequently, growth rate (Anjum et al., 2003a; Shao et al., 2008). This might be the reason for root length reduction.

Unlike drought stress, foliar application of SA and JA enhanced root length of *A. cepa* in presence of drought stress, which suggests that both of these growth regulators (GRs) reduced the effect of drought stress on root growth to a large extent. The presoaking of seeds in SA solution caused increase in root length in wheat cultivars
under drought stress (Heshmat et al., 2012). Similar results were observed in SA treated *Tagetus erecta* (Sandoval-Yapiz, 2004) and wheat (Singh and Usha, 2003) and these findings were strictly in tune with the observations made by Gutierrez-Coronado et al. (1998), where foliar application of SA significantly increased the length of roots in soybean. This root growth promoting domain of salicylic acid has now made it one of the most important, effective and cost beneficial phytohormone that has the potential to enhance the root growth in economically important vegetables and salads like *Daucus carota*, *Raphanus sativus* and *Beta vulgaris* (Aristeo-Cortes, 1998). In another study, it has been reported that drought stress inhibited root growth in *Allium sativum*; however, methyl jasmonate (ester of jasmonic acid) improved root growth when applied exogenously in presence of drought stress (Abumoslim and Javad, 2013). Similar results were observed under salt stress, one of the stresses causes water stress in plant, in JA treated soybean (Soad, 2007), pea (El Khallal, 2001), *Brassica napus* (Kaur et al., 2013) and pepper (Rezai et al., 2013). Sharma et al. (2013) reported that foliar spray with JA alone enhanced root length in *Cajanus cajan* when compared with untreated control.

**1.2. Shoot length**

The results indicate that drought stress caused a significant reduction in shoot length of *A. cepa* as compared to control. These results coincide with Heshmat et al. (2012), observed that drought stress caused a decrease in shoot length of wheat cultivars. Similar
results were observed in *Abelmoschus esculentus* (Sankar *et al.*, 2007 and 2008), *Vigna unguiculata* (Manivannan *et al.*, 2007a), soybean (*Zhang et al.*, 2004) and *Petroselinum crispum* (Petropoulos *et al.*, 2008) under drought stress condition. In soybean, the stem length was decreased under water deficit condition (Specht *et al.*, 2001). The plant height was reduced up to 25% in water stressed citrus seedlings (Wu *et al.*, 2000). Water stress greatly suppresses cell *expansion* and cell growth due to the low turgor pressure (Shao *et al.*, 2006). The reduction in plant height is associated with the decline in the cell enlargement and more leaf senescence under water stress (Bhatt and Srinivasa Rao, 2005). The reduction in shoot and root length in response to drought stress might be due to either decrease in cell elongation, cell turgor, cell volume and eventually cell growth (Banon *et al.*, 2006), and/or due to blocking up of xylem and phloem vessels thus hindering any translocation (Lavisolo and Schuber, 1998).

On the other hand, exogenous application of SA and JA enhanced shoot growth in drought stressed *A. cepa* as compared to drought stress alone. These results are in agreement with the observations of Hayat and Ahmad (2007), reported that under drought stress condition, SA treatment increased shoot length, shoot diameter and leaf number in ornamental plants such as, gloxinia and violet. In wheat cultivars, under drought stress, application of SA increased shoot length, shoot diameter and leaf area (Heshmat *et al.*, 2012). Similar results were found for shoot growth and height of the soybean plant (Gutierrez-Coronado *et al.*, 1998). It has been reported that SA
treatment increased shoot length and leaf number per plant in wheat seedlings (Hayat et al., 2005; Hussein et al., 2007). Further, growth of barley seedlings was promoted when sprayed with SA (Pancheva et al., 1996). Similar results were observed in maize (Khodary, 2004).

Sharma et al. (2013) reported that JA application promoted elongation of shoots in Cajanus cajan seedlings. Likewise, MeJA improved shoot length in pepper plants under salt stress (Rezai et al., 2013). Drought stress caused a significant reduction in shoot height of garlic, however, application of methyl jasmonate (MeJa) resulted in improvement of plant height in drought stressed garlic (Abumoslem and Javad, 2013). As reported by many authors, foliar application of MeJa increased shoot length in garlic and grapevine cultivars (Ravinkar et al., 1990 & 1993; Saimak et al., 2013). In a number of experiments, it has been noticed that foliar application of JA mitigated effects of salt stress by enhancing shoot length in soybean (Soad, 2007), pea (El Khallal, 2001), Brassica napas (Kaur et al., 2013).

**1.3. Whole plant fresh weight**

Drought stress significantly decreased whole plant fresh weight of A. cepa. Similar results were observed in sunflower (Tahir & Mehid, 2001), sugar beet genotypes (Mohammadian et al., 2005), soybean (Specht et al., 2001), Poncirus trifoliata seedlings (Wu et al., 2008), common bean and green gram (Webber et al., 2006), and Petroselinum crispum (Petropoulos et al., 2008) under drought stress condition. Defoliation originating from water stress in maize plants resulted in reduced biomass (Xin et al., 1998). However, it was also shown that
mild water stress decreased biomass production without a significant
effect on photosynthesis (Verelst et al., 2012). This demonstrates that
plants reduce their growth as an adaptation response to stress rather
than as a secondary consequence of resource limitations (Rollins et
al., 2013).

Foliar application of SA and JA enhanced whole plant fresh
weight of A. cepa in presence of drought stress. In a number of
experiments, many authors reported that drought stress caused a
serious reduction in fresh and dry weight in many plant species
however; reduction in this biomass was recovered by foliar application
of SA. For example SA application enhanced fresh and dry weight of
drought stressed barley (Habibi, 2012), corn, soybean (Khan et al.,
2003), wheat cultivars (El-Tayeb and Ahmed, 2010; Heshmat et al.,
2012), okra (Baghizadeh et al, 2009) and sahara plants (Jalal et al.,
2012). Drought stress caused reduction in fresh weight in garlic and
this damage was reduced by foliar application of MeJA when applied
to these drought stress plants (Abumoslem and Javad, 2013).
Similarly under salt stress, causing water shortage among plants,
fresh weight reduction was recovered by treating soybean plants with
JA (Soad, 2007). Treatment with MeJa, ester of JA, increased fresh
weight of pepper plants under salt stress (Rezai et al., 2013). Similar
results were observed, when tomato plant were sprayed with JA as
compared to untreated plants (Jennifer, 1999).
1.4. Whole plant dry weight

From the results it is clear that drought stress significantly decreased whole plant dry weight of *A. cepa* as compared to control. Tsialtas *et al.* (2001) reported that there was a one-third reduction in dry weight of the *Ziziphus rotundifolia* plants under drought conditions. Similar results were observed under drought stress in common bean (Yancy *et al.*, 1982), *Cyamopsis tetragonoloba* (Wang *et al.*, 2005) and spring wheat (Shao *et al.*, 2008a). A decrease in total dry matter may be due to the considerable decrease in plant growth, photosynthesis and canopy structure, as indicated by leaf senescence during water stress in *Abelmoschus esculentum* (Bhatt and Srinivasa Rao, 2005). Defoliation originating from water stress in maize plants resulted in reduced biomass (Xin *et al.*, 1998). Response of dry plant biomass compared to fresh plant biomass to water deficit is relatively lower and thereby dry mass/fresh mass ratio is used as a stress parameter at the plant level (Augé *et al.*, 2001; Zlatev and Lidon, 2012).

Exogenous application of SA and JA increased total dry weight of *A. cepa* under drought stress condition. These results are in agreement with those of Mohamed and Naglar (2010), who suggested that drought stress caused reduction of dry weight of shoot and root in wheat plants, while SA alleviated drought stress damages on dry weight of the same plant under drought stress. Similar results were reported in Okra plants (Baghizadeh *et al.*, 2009). SA when applied on *Rauwolfia serpentine* plants increased dry biomass under salinity
stress (Neelam and Rahul, 2012). Experiments on wheat seedlings under PEG induced oxidative stress, one of the stresses also caused by drought stress, shown that total biomass increased in response to SA treatments (Marcinska et al., 2013).

The results suggest that in response to JA treatment, dry weight of *A. cepa* increased under drought stress. Abumoslem and Javad (2013) reported that drought stress caused reduction in dry weight of garlic and this damage was reduced by foliar application of MeJA, ester of JA. Similarly, under salt stress, which causes water shortage among plants, fresh and dry weight reduction was recovered by treating soybean plants with JA (Soad, 2007). Treatment with MeJa increased dry weight of pepper plants under salt stress (Rezai et al., 2013). The enhancement in fresh and dry weight of drought stressed *A. cepa* in response to SA and JA may be due to improvement in shoot growth and relative water content which leads a higher photosynthesis rate and induction of antioxidant enzymes which prevent the cell damage by scavenging the reactive oxygen species. This fact is supported by Singh and Usha (2003), suggested that increase in dry mass of water stressed plants in response to SA may be related to the induction of antioxidant responses that protect the plant from damage.

2. **Yield parameters**

Drought stress significantly decreased number of cloves and total bulb weight of *A. cepa* as compared to control. Shao et al. (2008) reported that, seed yield and yield components are severely affected by
drought stress. Water stress reduced the seed weight and yield per plant in sunflower (Blumward et al., 2004). On the other hand, application of SA and JA to drought stressed A. cepa enhanced clove number and bulb weight. These results are consistent with the observations of Abumoslem and Javad (2013), that reduction in bulb growth and clove weight due to drought stress in garlic was alleviated by foliar spray with methyl jasmonate (MeJa), an ester of JA. Application of JA to soybean enhanced yield in the form of pod number and seed weight under salinity stress (Soad, 2007). Similar results were observed in MeJa treated soybean under drought stress condition (Anjum et al., 2011). Arfan et al. (2007) reported increased growth and grain yield in salt stressed wheat treated with SA. Similar results were obtained in SA treated wheat under drought stress (Heshmat et al., 2013). In addition, Shakirova et al. (2003) reported that, the treatment of wheat plants with salicylic acid (SA) increased the level of cell division within the apical meristem of seedling roots causing an increase in plant growth and elevated wheat productivity.

3. Relative water content

Drought stress caused a significant decrease in leaf relative water content (RWC) in A. cepa as compared to control. A decreased RWC was reported in Mungbean (Allahmomoradi et al., 2011), maize (Nayer and Reza, 2008) and wheat (Moaveni, 2011; Farshadfaret et al., 2011) under drought stress condition. Liu et al. (2006) reported a gradual decrease in RWC in wheat under PEG induced water stress.
RWC decreased with increase in the intensity of drought stress in barley (Gonzalez et al., 2008) and wheat (Shamsi, 2010).

It is obvious from the results that foliar spray of SA and JA increased RWC significantly in presence of drought stress. Reports suggest that drought stress resulted in a marked decrease in RWC; however, foliar application of SA enhanced RWC in leaves of Ctenanthe setosa (Kadioglu et al., 2011) and in barley (Habibi, 2012). Similarly, it was reported that exogenous SA treatment increased RWC in leaves of tomato (Tari et al., 2002) and Rauwolfia serpentina (Neelam and Rahul, 2012) under salt stress. Under PEG-induced drought stress, RWC decreased significantly in mustard seedlings; however, SA spray to these seedlings helped in maintaining water status by enhancing RWC to a marked level (Mahabub et al., 2013). Likewise, a sharp reduction in RWC was observed in soybean plants under drought stress, but application of MeJa to these plants recovered RWC to a significant level (Anjum et al., 2011).

4. Mineral content

The results suggest that drought stress significantly decreased potassium and calcium contents in root and shoot of A. cepa. However, foliar application of SA and JA enhanced the potassium and calcium contents under drought stress. These results are in accordance with the fact that drought stress decreased potassium and Calcium contents in wheat cultivars. However, exogenous application of SA enhanced these macronutrient contents in drought stressed wheat cultivars (Muhammad Waseem et al. 2006). Similarly drought
stress decreased mineral content (K, Mg, Ca, P etc) in *Fagus sylvatica* seedlings (Peuke *et al.*, 2010). Drought stress affected K, Mg and Ca content in wheat (El-Tayeb and Ahmed, 2010; Abdelkader *et al.*, 2012) and barley (El-Tayeb, 2005) while SA treatments increased the uptake of these minerals. Simeral results were observed in barley (El-Tayeb, 2005), soybean (Al-Hakimi, 2006), maize (Guner *et al.*, 2007), tomato (Szepesi *et al.*, 2000) and cucumber (Yildirim *et al.*, 2008) under drought and salt stress conditions. The reduction in mineral content uptake might be due the less nutrient availability and hindered membrane permeability and transport caused by drought stress.

5. **Photosynthetic pigments**

5.1. **Chlorophyll content**

Drought stress decreased chlorophyll content as compared to control. A reduction in chlorophyll content was reported in drought stressed cotton (Massacci *et al.*, 2008). The chlorophyll content decreased to a significant level at higher water deficits in sunflower plants (Kiani *et al.*, 2008) and in *Vaccinium myrtillus* (Tahkokorpi *et al.*, 2007). Reduction in chlorophyll content in plants such as *Paulownia imperialis* (Astorga and Melendez, 2010), bean (Beinsan *et al.*, 2003) and also *Carthamus tinctorius* (Siddiqi *et al.*, 2009) has been reported under drought stress. The decrease in chlorophyll content under drought stress has been considered a typical symptom of pigment photo-oxidation and chlorophyll degradation (Anjum *et al.*, 2011). Drought stress produced changes in photosynthetic pigments and components (Anjum *et al.*, 2003b), damaged photosynthetic
apparatus (Fu J. and Huang, 2001) and diminished activities of Calvin cycle enzymes, which are important causes of reduced crop yield (Monakhova and Chernyadèv, 2002). Another important effect that inhibits the growth and photosynthetic abilities of plants is the loss of balance between the production of reactive oxygen species and the antioxidant defense (Fu J. and Huang, 2001; Reddy et al., 2004), causing accumulation of reactive oxygen species which induces oxidative stress in proteins, membrane lipids and other cellular components.

In contrast to drought stress, treatment of SA and JA to drought stressed *A. cepa* increased chlorophyll content. It has been reported that pretreatment of *Brassica napus* with SA following drought stress caused significant increases in chlorophyll content (Maryam et al., 2012). There are several evidences about increasing chlorophyll content in SA treated plants species under drought stress such as, soybean (Zhao et al., 1995) and wheat cultivars (Horvath et al., 2007; Heshmat et al., 2012). Supplementation of SA increased chlorophyll a & b contents in drought stressed *Brassica juncea* seedlings (Mahabub et al., 2013). SA treatment reduced the stress-induced loss in chlorophyll content and enhanced rate of photosynthesis in *Rauwolfia serpentine* under drought stress (Neelam and Rahul, 2012). Similar results were observed by Khodary (2004) in maize under salinity stress.

Drought stress reduced chlorophyll contents in *Allium sativum* plants; however, application of MeJa mitigated this effect and
enhanced chlorophyll contents in presence of drought stress in these plants (Abumoslem and Javad, 2013). Similarly, exogenous foliar application of MeJa improved chlorophyll contents in grapevine cultivars (Saimak et al., 2013). Treatment with JA improved chlorophyll content in sweet basal under different regimes (Sorial and Gendy, 2010) and pigeon pea under oxidative stress (Sharma et al., 2013). As reported by many authors, application of JA enhanced chlorophyll contents and in turn rate of photosynthesis under salinity stress in Soybean (Soad, 2007), barley (Walia et al., 2007) and *Brassica napus* (Kaur et al., 2013). It has been suggested that JA treatment increases active cytokinin concentration which enhanced chlorophyll accumulation in potato plants (Kovac and Ravnikar, 1994). Rezai et al. (2013) suggested that reduction in chlorophyll content due to salt stress was mitigated by exogenous application of MeJa in pepper plants.

### 5.2. Carotenoid content

From the results it is clear that drought stress reduced carotenoid content as compared to control. However, application of SA and JA enhanced carotenoid content in leaves of drought stressed *A. cepa*. it has been reported that drought stress reduced carotenoid content, however, foliar application of SA enhanced carotenoid content in drought stressed *Ctenanthe setosa* plants (Kadioglu et al., 2011), wheat seedlings (Heshmat et al., 2012) and *Brassica napus* plants (Maryam et al., 2012). SA application activated the synthesis of carotenoids in wheat and moong seedlings (Moharekar et al., 2003).
Similar reports has been observed when *Rauwolfia serpentina* plants were sprayed with SA under salinity stress (Neelam and Rahul, 2012). Enhancement of carotenoid content by SA may be related to the protecting role of these compounds in photosynthetic machinery. Carotenoid could scavenge or quench the singlet oxygen and protect the chloroplast from lipid peroxidation and oxidative damages (Loggini, 1999).

Methyl jasmonate, methyl ester of jasmonic acid, showed positive effects by enhancing carotenoid content when applied exogenously to garlic plants under drought stress (Abumoslem and Javad, 2013). Similarly, foliar application of JA improved carotenoid synthesis in presence of drought stress in marigold plants (Sedghi *et al*., 2012). It was further reported that JA treatment significantly increased carotenoid content in soybean under salinity stress (Soad, 2007). Similar results were observed in *Cajanus cajan* plants treated with JA under copper induced oxidative stress (Sharma *et al*., 2013).

6. Biochemical parameters

6.1. Protein content

Drought stress significantly decreased protein content both in root and shoot of *A. cepa*. These results are consistent with those observed in *Phaseolus vulgaris* (Ramos *et al*., 1999), sunflower (Rodriguez *et al*., 2002) and chickpea (Mafakheri *et al*., 2010). The reduction in quantity of soluble proteins observed in present experiment can be related to the reduced rate of protein biosynthesis and increased breakdown of protein under limited environment (Chen
et al., 1999). Under water limited conditions, plants activate the pathway of protein breakdown, because the plants use the proteins for the synthesis of nitrogen compounds as amino acids that might support the plant osmotic adjustment (Sankar et al., 2007). As suggested by earlier workers, protein degradation might be the result of increased activity of protease or other catabolic enzymes, which activates under drought stress, or due to fragmentation of proteins due to toxic effects of reactive oxygen species resulting in reduced protein content (Davies 1987). A decrease in the protein concentration would be a typical symptom of oxidative stress and has frequently been observed in drought-stressed plants (Seel et al., 1992; Moran et al., 1994).

The results suggest that application of JA and SA enhanced the protein content in *Allium cepa* under drought stress. Heshmat et al. (2012) reported that protein content decreased in wheat cultivars under drought stress, when SA was applied to these drought stressed wheat plants, protein content was found increased significantly. Similar results were observed in SA treated barley (El Tayeb, 2005), sunflower (El Tayeb et al., 2006), soybean (Al Hakami, 2006), maize (Gunes et al., 2007), wheat (El Tayeb and Ahmed, 2010) and *Satureja hortensis* under drought stress conditions. These results were further supported by Jalal et al. (2012) who reported that drought stress decreased protein content considerably, but SA treatment reduced this negative effect and enhanced protein content in shara plants. Similarly, Soad (2007) reported that foliar application of JA enhanced
protein content in soybean under salt stress. In another report it was suggested that JA treatment, both in presence or absence of oxidative stress, significantly increased protein content in *Cajanus cajan* (Sharma *et al*., 2013). Similar results were found in JA treated plants such as, soybean (Anderson, 1991), rice (Rakwl and Komastu. 2001) and peanut (Kumari *et al*, 2006). It was further reported that JA application improved protein content in maize seedlings under pathogen induced biotic stress (Saghar and Maryam, 2011).

### 6.2. Amino acid content

The results obtained indicate that the accumulation of amino acid increased under drought stress as compared to control in root and shoot of *Allium cepa* on all growth stages. These results are in line with those observed under drought stress in *Arachis hypogaea* (Asha and Rao, 2002), sorghum (Yadav *et al*., 2005), *Phragmites australis* (Pagter *et al*., 2005), pepper (Nath *et al*., 2005), *Radix astragali* (Tan *et al*., 2006), and *Marsh* grasses (Maricle *et al*., 2008). Strogonov (1964) attributed that the accumulation of amino acids may be due to the hydrolysis of protein and also accumulated amino acid may be occurring in response to the change in osmotic adjustment of their cellular contents (Greenway and Munns, 1980 and Shao *et al*., 2007). Amino acid accumulation plays a very important role in drought tolerance, probably through osmotic adjustment in different plant species, such as *Radix astragali* (Tan *et al*., 2006).

From results it is clear that foliar application of JA and SA further increased the amino acid content to a significant level both in
root and shoot of *Allium cepa* under drought stress. These results were supported by El Tayeb, (2005) which suggests that under stress conditions, SA treated plants exhibited a higher accumulation of free amino acids in barley plants. Similarly foliar application of JA markedly enhanced free amino acid content in salt stressed soybean plants (Soad, 2007). JA treated pea plants exhibited higher accumulation of amino acid content (El Khallal, 2001).

6.3. Proline content

Under drought stress the proline accumulation increased in the root and shoot of *Allium cepa* on all growth stages. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce cell injury. These results are consistent with those of Anjum *et al.* (2011a) who observed that progressive drought stress induced a considerable accumulation of proline in water stressed maize plants. Similar results were observed under drought stress in Sorghum (Yadav *et al.*, 2005), Bell pepper (Amarjit *et al.*, 2005), *Gossypium hirsutum* (Ronde *et al.*, 1999), wheat (Hamada, 2000) and *Cyamopsis tetragonoloba* (Shubhra *et al.*, 2003). Proline accumulation in plants might act as a scavenger of ROS and acting as an osmo-protectant to reduce water potential which in turn helps to retain water content inside the cell. The reduced proline oxidase may be the reason for increasing proline accumulation. Under stressful conditions, proline accumulation supplies energy for the growth and survival and thereby helps the plant to tolerate stress (Jaleel *et al.*, 2007d). Proline, as an osmo-protectant compound, plays a major role
in osmo-regulation and osmo-tolerance (Demir, 2000). However, its
definite role in exerting stress resistance continues to be a debate
(Demiral and Turkan, 2006).

Foliar spray of SA and JA markedly increased the proline
accumulation in root and shoot of the drought stressed Allium cepa
plants. Kadioglu et al. (2011) suggested that there was a substantial
increase in proline content in SA treated Ctenanthe setosa plants
under drought stress. Similar results were observed when SA was
exogenously applied to drought stressed plants such as Brassica
napus (Maryam et al., 2012), okra (Baghizadeh et al., 2009), wheat (El
Tayeb and Ahmed, 2010; Heshmat et al., 2012) and Satureja hortensis
(Yazdanpanah et al., 2011). It was further reported that SA treatment
increased proline synthesis under salt stress in R. serpentina (Neelam
and Rahul, 2012) and wheat seedlings (Shakirova et al., 2003).
Shakirova et al. (2005) suggested that SA treatment induced changes
in phytohormones and increase the ABA level, which is responsible for
the induction of proline biosynthesis under stress condition. There
was a steady increase in proline content in soybean under drought
stress; however, MeJa application further enhanced the proline
accumulation in these drought stressed plants (Anjum et al., 2011).
Foliar application of JA significantly increased proline content in salt
stressed pea (El Khallal, 2001) and soybean (Soad, 2007). There are
many reports that increased proline content was observed by foliar
application of JA in Cajanus cajan (Sharma et al., 2013), pear (Gao et
al., 2004) and barley (Walter et al., 2002) under various environmental
stresses. Moreover, proline accumulation can be explained by the higher inhibitory rate of proline oxidase. A significant higher elevation in γ-glutamyl kinase activity (proline synthesis) associated with inhibition of proline oxidase activity (proline oxidation) is the reason for the higher level of free proline accumulation (Neelam and Rahul, 2012). It has been reported that proline accumulation under drought stress can be attributed to the increased level of γ-glutamyl kinase activity (Manivannan et al., 2008). The decrease in proline oxidase activity with increasing rate of γ-glutamyl kinase activity might be the reason for higher proline accumulation in drought-stressed *A. cepa*.

### 6.4. Glycine betaine

The results revealed that glycine betaine accumulation increased under drought stress in both root and shoot of *Allium cepa*. These results are in accordance with the observations of Tan *et al.* (2006) that glycine betaine content increased under drought stress in *Radix astragali*. There are similar evidences that glycine betaine content increased under drought stress in barley (Nakamura, 2001) and in higher plants (Jun *et al.*, 2000). Glycine betaine is considered to be one of the most abundant quaternary ammonium compounds produced in higher plants under stressful environment (Yang *et al.*, 2003).

Foliar application of SA and JA further boosted the glycine betaine accumulation in drought stressed *Allium cepa* plants. These results are in line with reports which suggested that foliar application of SA enhanced glycine betaine content in *Rauwolfia serpentina* under
salt stress (Neelam and Rahul, 2012). Likewise, as reported by Gao et al. (2004), JA is able to elicit betaine accumulation in pear.

6.5. Total soluble sugar content

Drought stress caused a significant increase in total soluble sugar content in all parts of Allium cepa when compared with control plants. Similar reports were observed under drought stress in Zea mays cultivars (Nayer and Reza, 2008), Oryza sativa cultivars (Mostajeran and Rahimi – Eichi, 2009) and tomato (Mohamed et al., 2011). The reducing sugar content increased in parallel with the invertase activity under drought stress in Alfalfa seedlings (Zeid and Shedeed, 2006). The increased total sugar content might be due to the degradation of starch under drought stress.

It is evident from the results that exogenous application of SA and JA further increased total soluble sugar content in Allium cepa in presence of drought stress. These results are in agreement with observations of Heshmat et al. (2012) that foliar application of SA caused a noticeable increase in soluble sugar content in different drought stressed wheat cultivars. Similar results were found in SA treated okra plants (Baghizadeh et al., 2009), wheat (El Tayeb and Ahmed, 2010) and Satureja hortensis (Yazdanpanah et al., 2011) under drought stress. In another investigation, El Tayeb et al. (2006) observed that SA treatment increased total sugar content in sunflower under Copper induced oxidative stress. Similar results were observed by Khodary (2004) and El Tayeb (2005) in SA treated maize and barley plants respectively under salinity stress. Likewise, application of JA
enhanced total sugar and carbohydrate content in *B. napus* (Kaur *et al.*, 2013) and soybean (Soad, 2007) under salt stress. There was a significant increase in total sugar content in JA treated maize plants in absence or presence of pathogen induced biotic stress (Saghar and Maryam, 2011).

6.6. Sucrose content

The results suggested that drought stress increased sucrose content significantly in root and shoot of *Allium cepa*. These results are in line with Fulia Liu *et al.* (2004), suggested that the sucrose concentration was higher in drought stressed soybean. Similar result was found in epiphytic orchid under drought stress (Giulio *et al.*, 2001). Increasing evidence shows that sucrose may play non-nutritive role as a regulator of cellular metabolism possibly by acting at the level of gene expression (Jang and Sheem, 1994). The increased levels in sucrose content may be the cause of conversion of starch into sucrose.

When *Allium cepa* plants were treated with SA and JA in presence of drought stress, sucrose content was markedly increased in both root and shoot. Similar results were found in SA treated maize and sunflower plants (Khodary, 2004; El-Tayeb *et al.*, 2006). It has been further reported that sucrose content increased under drought stress in wheat cultivars, but SA treatment caused an additional increase in sucrose content in presence of drought stress (Heshmat *et al*, 2012). Application of JA enhanced total carbohydrate content in soybean (Soad, 2007) and *B. napus* (Kaur *et al.*, 2013).


6.7. Starch content

The results indicate that starch content decreased under drought stress in the root and shoot Allium cepa. However, foliar application of SA and JA enhanced starch content in presence of drought stress. These results coincide with Bianchi et al. (1992), reported decreased starch content in Craterostigma plantagineum under drought stress. Similar results were observed in Ramonda and Haberlea (Muller et al., 1997) and grapevine (Patakas and Noitsakis, 2001) under drought stress. Starch content significantly decreased in both roots and shoots of maize cultivars under water-deficit stress (Mohammandkhani and Heidai, 2008). Starch degradation is a common observation in the leaves of water stressed plants (Stewart, 1971). The decreased total carbon flux might be concern for greater decline in starch than sucrose in leaf (Lawlor, 1995). Work on excised bean leaves also suggested that wilting caused starch to be converted to sucrose (Stewart, 1971). The reason for starch degradation might be to retain the water content by decreasing the water potential by conversion of starch into sucrose and other sugars which act as osmolyte.

The results further revealed that foliar application of SA and JA markedly improved starch content in root and shoot of drought stressed A. cepa plants. This is supported by the report that drought stress induced reduction in carbohydrate content in wheat cultivars was mitigated by exogenous foliar application of SA (Heshmat et al., 2012). Similar results were observed in SA treated maize (Khodary,
and barley plants (El Tayeb, 2005) under salt stress. It has been reported that SA treatment enhanced carbohydrate content in sunflower under Copper induced oxidative stress (El Tayeb et al., 2006). It was further reported that foliar application of JA accumulated carbohydrate content in Salt stressed pea (El Khallal, 2001) and soybean (Soad, 2007). Similar reports were noticed in JA treated *B. napus* seedlings under salt stress (Kaur et al., 2013).

**7. Proline metabolism enzymes**

**7.1. γ-glutamyl kinase activity**

It is clear from the results that the rate of γ-glutamyl kinase activity significantly increased in root and shoot of drought stressed *A. cepa* plants as compared to control. Similar results were reported in drought stressed plants such as *Abelmoscus esculentus* (Sankar et al., 2007), *Catharanthus roseus* (Jaleel et al., 2008) and *Helianthus annuus* (Manivannan et al., 2008). The proline accumulation in drought stressed *Catharanthus roseus* can be attributed to the increased level of γ-glutamyl kinase activity (Sakamoto et al., 1998). The γ-glutamyl kinase is an important regulating enzyme in the synthesis of proline. The proline accumulation in drought stressed *A. cepa* plants can be correlated with the increased level of γ-glutamyl kinase activity. This may be supported by the report that induction of proline accumulation may be due to an activation of proline synthesis through glutamate pathway involving γ-glutamyl kinase, glutamyl phosphate reductase and Δ1-pyroline-5-carboxylate reductase.
activities in peanut (Girija et al., 2002) and in tomato (Fujita et al., 2003).

The results again suggest that exogenous application of SA and JA further increased the rate of \( \gamma \)-glutamyl kinase activity in presence of drought stress in *A. cepa*. It has been reported that different growth regulators increased \( \gamma \)-glutamyl kinase activity in pea (Wang and Lin, 1992) and wheat plants (Kraus and Fletcher, 1994). Similar results were found in jack pine under drought stress (Marshall et al., 1991). Exogenous SA application increased the \( \gamma \)-glutamyl kinase activity in *Rauwolfia serpentina* under salt stress (Neelam and Rahul, 2012).

### 7.2. Proline oxidase

Drought stress decreased the rate of proline oxidase activity of *A. cepa* in both root and shoot. These results coincide with the report that a sharp reduction in proline oxidation was observed under water stress in bean (Flowers and Hanson, 1969) and *Zea mays* (Sells and Koeppe, 1981). It is further reported that drought stress caused a sharp decrease in proline oxidase activity in bhindi (Sankar et al., 2007), spinach (Huang and Cavalieri, 1979), mulberry (Veeranjaneyulu and Kumari, 1989), cassava (Sundaresan and Sudhakaran, 2006) and Sunflower (Manivannan et al., 2007). The similar reports coincide under water stress in tomato (Fujita et al., 2003) and *Arabidopsis esculentus* (Hong et al, 2000). Proline oxidase converts proline to glutamate. Thus this enzyme also influences the level of free proline. The \( \Delta^1 \)-pyrrolle-5-carboxylate synthetase is the rate-limiting enzyme in proline biosynthesis in plants and is subjected
to feedback inhibition by proline. It has been suggested that the feedback regulation of P5CS is lost in plants under stress conditions (Hong et al., 2000). The results indicate that foliar application of SA and JA to *A. cepa* plants increased proline oxidase activity as compared to drought stress alone, but it does not exceed the control. Exogenous SA application altered proline oxidase activity in *Rauwolfia serpentena* under salt stress (Neelam and Rahul, 2012).

8. Non-enzymatic antioxidants

8.1. α-tocopherol content

It is clear from the results that α-tocopherol content increased under drought stress in root and shoot of *A. cepa*. Similar results were observed in turf grass under water stress (Zhang and Schmidt, 2000). Synthesis of low-molecular-weight antioxidant, α-tocopherol, has been reported in sorghum seedlings (Zhang and Kirkham, 1996), wheat (Loggini et al., 1999, Carlos et al., 1999), grasses (Fu and Huang, 2001), maize (Jiang and Zhang, 2002), rice (Boo and Jung, 1999; Srivalli et al., 2003) and apple tree (Sircelj et al., 2005) subjected to drought stress. The active oxygen species formed at the membrane of wheat leaves under drought stress was efficiently removed upon dehydration with increase in the α-tocopherol and β-carotene (Bartoli et al., 1999). Perl et al. (1993) noted that water stress causes an accumulation of reactive oxygen species in the chloroplasts. This may result in an increase of α-tocopherol, which quenches oxygen radicals within the membrane and terminates chain reaction that cause oxidative damage. The α-tocopherol is consumed predominantly as a
radical scavenging antioxidant against lipid peroxidation (Bartoli et al., 1999). Foliar treatment with SA and JA further enhanced the α-tocopherol content in drought stressed A. cepa on all growth stages. This is supported by the report that salicylic acid application increased α-tocopherol content in the leaves of drought stressed Ctenanthe setosa plants (Kadioglu et al., 2011).

8.2. Ascorbic acid content

Ascorbic acid content increased in root and shoot of A. cepa under drought stress when compared with control. These results are in line with the report that water stress significantly increased ascorbic acid concentration in turf grass (Zhang and Schmidt, 2000). Similar results were observed in sorghum seedlings (Zhang and Kirkham, 1996); Triticum aestivum (Carlos et al., 1999); rice (Srivalli et al., 2003); apple tree (Sircelj et al., 2005) and in Poncirus trifoliata (Wu et al., 2006) under drought stress. Ascorbic acid is a very important reducing substrate for H₂O₂ detoxification in photosynthetic organisms (Lee et al., 2003) and participates in the removal of H₂O₂ as a substrate of ascorbate peroxidase. It has been reported that ascorbic acid content in the leaves of Withania somnifera increased under drought stress (Jaleel, 2009). Pradeep et al. (2011) reported similar results in drought stressed chickpea.

The foliar application of SA and JA further increased the concentration of ascorbic acid in root and shoot of A. cepa in presence of drought stress. These results are in accordance with following reports. Kadioglu et al. (2011) reported that accumulation of ascorbic
acid content in *Ctenanthe setosa* plants was further enhanced by exogenous application of SA as compared to drought stressed control. Furthermore, ascorbic acid content was found increased when mustard seedlings were sprayed with SA under drought stress (Mahabub *et al.*, 2013). These reports are supported by Rao *et al.* (1997) and Dat *et al.* (1998), as they found similar results in many plants. It was further reported that MeJa, an ester of JA, under stress conditions, improved accumulation of ascorbic acid in *Arabidopsis thaliana* (Sasaki *et al.*, 2005) and soybean (Keramat *et al.*, 2009). It has been suggested that JA play an important role in ascorbate metabolism (Li *et al.*, 1998; Ai *et al.*, 2008).

### 8.3. Reduced glutathione content

The results suggest that reduced glutathione (GSH) content increased under drought stress in root and shoot of *A. cepa* on all growth stages. Similar results were observed in wheat (Loggini *et al.*, 1999) and apple (Sircelj *et al.*, 2005) under drought stress condition. Drought stress caused a significant increase in reduced glutathione content in *sorghum* (Zhang and Kirkham, 1996), rice (Srivalli *et al.*, 2003), *Portulaca oleracea* (Yazici *et al.*, 2007) and *Ctenanthe setosa* (Kadioglu *et al.*, 2011). It has been suggested that reduced glutathione plays an important role in the regeneration of glutathione and thus protects against oxidative stress also by maintaining the ascorbic acid pool, as in transgenic tobacco plants (Ding *et al.*, 2009). Glutathione take part in the control of H$_2$O$_2$ levels (Foyer and Noctor, 2001). The change in the ratio of its reduced (GSH) to oxidized (GSSG) form
during the degradation of $\text{H}_2\text{O}_2$ is important in certain redox signaling pathways (Pastori and Foyer, 2002; Noctor et al., 2002).

Foliar application of SA and JA further increased the reduced glutathione content in drought stressed A. cepa. These results are in accordance with Mahabub et al. (2013), who ascertained that PEG induced drought stress, caused a significant increase in GSH content in mustard seedlings. When these drought stressed seedlings were supplemented with SA, GSH content was further enhanced to a marked level. Similar results were reported in wheat and rape-seed seedlings (Shao et al., 2005; Hasanuzzaman and Fujita, 2011). It has been further reported that reduced glutathione levels were found increased in SA treated mustard seedlings under heat stress (Dat et al., 1998). These reports are further supported by Rao et al. (1997), suggested that GSH levels were influenced by SA treatment under oxidative stress. Salicylic acid application increased reduced glutathione (GSH) in the leaves of drought stressed Ctenanthe setosa plants (Kadioglu et al., 2011).

9. **Enzymatic antioxidants**

9.1. **Superoxide dismutase activity**

Drought stress increased the rate of SOD activity in root and shoot of A. cepa. These results are consistent with other studies reporting the increased SOD activity in response to drought stress in pea (Malecka et al., 2001), wheat (Csiszar et al. 2005), liquorice (Pan et al., 2006), sunflower (Gunes et al., 2008) and poplar (Xiao et al., 2008). Similar results were observed under drought stress in maize
(Pastori et al., 2000; Jiang and Zhang, 2002), *Euphorbia esula* (Davis and Swanson, 2001), *Cassia angustifolia* (Agarwal and Pandey, 2003), wheat (Singh and Usha, 2003; Shao et al., 2005), rice (Wang et al., 2005). The high SOD activity has been associated with stress tolerance in plants because it neutralizes the activity of $O_2^-$ which was over produced under stress (Bowler et al., 1992). There are few more reports as supporting evidences, who suggested that drought stress caused a significant increase in SOD activity in *Triticum aestivum* (Rachana et al., 2011; Bano et al., 2012), barley (Mortaza et al., 2012), spring barley (Ghader Habibi, 2013) and melon seedlings (Musa et al., 2013).

Results suggest that SOD activity was further enhanced by foliar application of SA and JA when applied to drought stressed *A. cepa*. Plant growth regulator like SA increased the activity of SOD in drought stressed *Ctenanthe setosa* plants (Kadioglu et al., 2011). Ghader Habibi (2012) found similar results in PEG induced drought stressed barley plants supplemented with SA. These results are consistent with those found by Hayat et al. (2008 & 2010) in tomato plants, admitted that SA enhanced the SOD activity under drought stress. There was a significant increase in SOD activity under drought stress in marigold; however, JA treatment further enhanced SOD activity to a marked level (Sedghi et al., 2012). In another report, it was suggested that SOD activity significantly increased in MeJa treated soybean plants under drought stress (Anjum et al., 2011). Under oxidative stress, similar reports were observed in soybean when
treated with MeJa (Keramat et al., 2009). These results are further supported by Sharma et al. (2013), who suggested that foliar application of JA enhanced rate of SOD activity in Cajanus cajan both in presence or absence of oxidative stress.

**9.2. Peroxidase activity**

The rate of peroxidase activity increased under drought stress as compared to control. These results are supported by many evidences that suggests increased peroxidase activity under drought stress in soybean plants (Zhang et al., 2006), wheat (Lin and Wang, 2002; Gong et al., 2005), Pinus halepensis (Alonso et al., 2001), Hordeum vulgare (Acar et al., 2001), soybean (Heerden and Kruger, 2002), Doritaenopsis (Cui et al., 2004) and rice (Guo et al., 2006). Peroxidase catalyzes the dehydrogenation of structurally diverse phenolic and endolic substances by H₂O₂ and thus often regarded as antioxidant enzyme, protecting cells from the destructive influence of H₂O₂ and derived oxygen species (Shigeoka et al., 2002). Plant peroxidases are commonly known for their capability to reduce H₂O₂ to water at the expense of hydrogen donors.

The results indicate that treatment with SA and JA further increased POD activity in A. cepa plants exposed to drought stress. These results coincide with those observed by Hayat et al. (2008) that under drought stress POD activity increased in tomato, but SA treatment accelerated POD activity further under drought stress. Similar reports were observed by Habibi (2012) when barley plants were sprayed by SA under drought stress. Increased POD activity was
observed under drought stress in soybean plants, but MeJa treatment further enhanced POD activity in these stressed plants (Anjum et al., 2011). Under different stress conditions, it was reported that MeJa treatment enhanced POD activity in *A. thaliana* (Jung, 2004), soybean (Keramat *et al*., 2009), pigeon pea (Sharma *et al*., 2013) and grapevine (Saimak *et al*., 2013). On the other hand, there were found no significant differences in POD activity between drought stressed and JA treated marigold plants (Sedghi *et al*., 2012).

### 9.3. Catalase activity

Increased catalase activity was recorded in the root and shoot of *A. cepa* under drought stress. This is in agreement with many studies which postulate that catalase activity increased under drought stress in *Oryza sativa* (Chandrashekara Reddy *et al*., 1998), maize (Pastori *et al*., 2000), *Zea mays* (Jiang and Zhang, 2002), *Allium schoenoprasum* (Egert and Tevini, 2002), wheat (Dalmia and Sawhney, 2004; Shao *et al*., 2005) and *P. acutifolius* (Turkan *et al*., 2005). An increase in catalase activity was reported in higher plants under drought stress (Reddy *et al*., 2004). Similar results were found in *Lotus corniculatus* (Borsani *et al*., 2001) and rice (Wang *et al*., 2005). The increased activity of catalase might be due to the enhanced superoxide dismutase activity (Casano *et al*., 1999). Hydrogen peroxide is broken down to water by CAT and POD (Asada, 1992).

Peroxidase activity was further enhanced in drought stressed *A. cepa* by foliar application of SA and JA. These results are in line with the following reports, that exogenous application of SA significantly
increased rate of CAT activity in drought stressed tomato (Hayat et al., 2008), *Ctenanthe setosa* plants (Kadioglu et al., 2011) and mustard seedlings (Mahabub et al., 2013). There are evidences, supporting above reports, which suggest that application of SA improved CAT activity to a significant level in black cumin (Kabiri et al., 2012) and barley (Habibi, 2012) under drought stress. There are few reports about inhibition of CAT activity by SA treatment (Janda et al. 2003; Shakirova, 2007). This is not the case in all plant species, as Farooq et al. (2009a) reported that CAT inhibition by SA cannot be validated in all plants. Similarly, application of JA in presence of drought stress significantly enhanced CAT activity in marigold (Sedghi et al., 2012) and soybean (Anjum et al, 2011). It has been further reported that foliar application of MeJa significantly increased CAT activity in *A. thaliana* (Jung, 2004), soybean (Karamat et al., 2009), maize (Saghar and Maryam, 2011) and grapevine cultivars (Saimak et al., 2013) under different stress conditions.

### 9.4. Ascorbate peroxidase

The results suggest that APX activity increased under drought stress in root and shoot of *A. cepa* plants when compare with control. These results are consistent with many reports which suggest that ascorbate peroxide activity increased under drought stress in *Euphorbia escula* (Davis and Swanson, 2001), *Zea mays* (Jiang and Zhang, 2002), wheat (Dalmia and Sawhney, 2004) and *P. accutifolius* (Turkan et al., 2005). Similar results were observed in *Vigna* plants (Manivannan et al., 2007a), soybean (Heerden and Kruger, 2002) and
in *Catharanthus roseus* (Jaleel *et al.*, 2007d). Sofo *et al.* (2004) observed that leaves of olive trees experiencing severe drought stress showed considerably more APX activity than the roots. Ascorbate peroxidase reduces H$_2$O$_2$ to H$_2$O by using ascorbate as reducing agent, thus protects the plant (Asada, 1992; Meloni *et al.*, 2003).

Foliar application of SA and JA further increased APX activity in drought stressed *A. cepa* in both root and shoot. These results are supported by many reports that confer SA treatment enhanced APX activity under drought stress condition such as in *C. setosa* (Kadioglu *et al.*, 2011), black cumin (Kabiri *et al.*, 2012), barley (Habibi, 2012) and mustard seedlings (Mahabub *et al.*, 2013). It has been reported that SA treatment enhanced the activity of APX (Janda *et al.*, 2003; Shakirova, 2007). Similar reports were observed by Krantev *et al.* (2008) in SA treated maize plants. In an experiment, Saimak *et al.* (2013) observed that foliar application MeJa enhanced APX activity in grapevine cultivars. Similar reports were noticed in MeJa treated *A. thaliana* (Jung, 2004), maize (Norastehnia and Asghari, 2006), *Panax ginseng* (Mohammad *et al.*, 2007) and soybean (Keramat *et al.*, 2009).

10. Lipid peroxidation

The results revealed that drought stress caused a significant increase in lipid peroxidation by increasing MDA content both in root and shoot of *A. cepa*. This is in accordance with the studies of Yang and Miao (2010), which confer increments of the MDA and H$_2$O$_2$ concentrations in the water-stressed *P. cathayana* and *P. kangdingensis*. The levels of lipid peroxidation in leaves of *Pisum*
sativum increased two to four fold with an increase in drought stress, and this was highly correlated with protein peroxidation (Moran et al., 1994). Drought-induced overproduction of ROS increases the content of malondialdehyde (MDA). The content of MDA has been considered an indicator of oxidative damage (Moller et al., 2007). MDA is considered as a suitable marker for membrane lipid peroxidation. A decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS. Furthermore, lipid peroxidation is an indicator of the prevalence of free radical reaction in tissues (Daneshmand et al., 2010).

Application of SA and JA reduced lipid peroxidation by decreasing the MDA content in drought stressed A. cepa plants. This is supported by the report that MDA content increased significantly in B. napus plants under drought stress; however, SA treatment protected these drought stressed plants by reducing MDA content (Maryam et al., 2012). Drought stress increased lipid peroxidation, while SA treatment prevented lipid peroxidation by decreasing MDA content under drought stress in C. setosa (Kadioglu et al., 2011), Satureja hortensis (Yazdanpanah et al., 2011), black cumin (Kabiri et al., 2012) and mustard seedlings (Mahabub et al., 2013). Similar results were observed in SA treated barley under salt stress (El Tayeb, 2005). It is further suggested that SA could decline lipid peroxidation through the inhibition of lipoxygenase activity and decline of H$_2$O$_2$ content and have maintain the cell membrane integrity under stress conditions (Hayat et al., 2007). MDA was found increased under
drought stress in Marigold, while JA application declined MDA content and hence prevent lipid peroxidation in Marigold (Sedghi et al., 2012). Similar results were noticed in MeJa treated strawberry (Wang, 1999), soybean (Anjum et al., 2011) and banana (Mahmood et al., 2012) under drought stress. It has been reported that MeJa application under cadmium induced oxidative stress in soybean alleviated damage caused by excessive lipid peroxidation by reducing MDA content formation (Keramat et al., 2009). Similar results were observed in JA treated pigeon pea under copper induced oxidative stress (Sharma et al., 2013).

11. Hydrogen peroxide content

The results indicate that drought stress caused a significant increase in $\text{H}_2\text{O}_2$ content in root and shoot of $\textit{A. cepa}$. However, foliar application of SA and JA to the drought stressed $\textit{A. cepa}$ plants noticeably reduced $\text{H}_2\text{O}_2$ content. These results are consistent with many reports which claimed that SA application declined the rate of $\text{H}_2\text{O}_2$ production caused by drought stress in rice (Ganesam and Thomson, 2001), $\textit{C. setosa}$ (Kadioglu et al., 2011) and black cumin ($\textit{Kabiri et al.}$, 2012). Drought stress caused a remarkable increase in $\text{H}_2\text{O}_2$ in mustard seedlings, while spraying with SA prevented the accumulation of excessive $\text{H}_2\text{O}_2$ by up-regulation of $\text{H}_2\text{O}_2$ scavenging enzymes (Mahabub et al., 2013). Similar results were noticed in SA treated rice seedlings (Panda and Patra, 2007). Likewise, application of MeJa removed the adverse effect of drought stress by inhibited formation of excessive $\text{H}_2\text{O}_2$ in strawberry (Wang, 1999) and banana.
(Mahmood et al., 2012). Similar results were observed by Keramat et al. (2009) in soybean when plants were treated with MeJa under Cd induced oxidative stress.