Drought is one of the most important environmental stresses affects the plant growth and crop production severity. In this experiment, the effect of osmotic stress on proline and soluble sugars content was investigated in two canola cultivars (Brassica napus L.) SLM046 is drought tolerant and Hyola 308 is drought sensitive. To establish water deficit, the 12 days old canola seedlings were placed in half strength Hoagland solution containing different levels of PEG 6000 (0, 5%, 10% and 15%) for 24 hours. The results indicated that water stress increased proline content in shoots and roots of the studied cultivars; however, in stress conditions, amount of proline in SLM046 was higher than that in Hyola 308. On the other hand, drought increased glucose, mannose and threonine contents in the both tissues of canola cultivars, but contents of these soluble sugars in SLM046 was significantly higher than that of Hyola 308.

INTRODUCTION

Drought, high and low temperature and soil salinity not only affects germination and plant growth but also produce adversely such as pests, disease and weed (Manivannan, 2007). More than 45% of agricultural lands are subject to drought constantly and 38% of population in the world lives there. Therefore, more endeavors would be done toward more cropping production in low water (Vinocur and Altman, 2005). In other words, we should produce more production based on each one drop of water. Water shortage (which is called drought) can be defined as lack of enough and essential moisture for a plant in order to have a normal growth and completing life cycle. As plants cannot escape of various environmental stresses, they would need mechanisms for identifying stresses and responding them (Ahmad and Sharma, 2010). Osmoregulation is one of these mechanisms. Osmotic regulation is a kind of compatibility with water deficit. By concentration of dissolved substances into cells, it can be led to protect turgescence of cells and process depending on it in low water potentials. This regulation is done by more production of various organic such as proline, protein, betaine, soluble sugars in the roots and shoots. Soluble sugar acts as osmotic regulators, stabilizer of cell membranes and preserving of cells turgescence (Sadiqov et al 2002). Actually, osmoregulation is done better in plants which soluble sugars gather up due to drought stress. Proline is another osmoregulator. Amino acid of proline is identified in many plants. They usually accumulate by large amount in response to environmental stresses (Mostajeran and Rahimi-Eichi, 2009). Synthesizing proline after finishing stress conditions may provides required factors of mitochondrial oxidative phosphorylation and producing ATP for repairing damages due to stress. Main role of proline has been approved in plants exposed to drought and its accumulation in plants suffering stress is more than sensitive numbers. Amount of several other amino acids boost due to various stresses such as drought and salinity; however, the amount of these changes is not comparable with those changes which has been happened in prolin and reaches to a high level after a short time. In vitro conditions, polyethylene glycol (PEG) is used for observing damages due to drought stress (Ashraf and Foolad 2007). PEG is molecules with high weight that decrease water potential in a method similar to drought. As the osmotic composition is not absorbed by cells easily, it does not affect on ionic composition. In other words, PEG cannot pass through the cell membrane (while sucrose passes through the cell membrane). So it is not toxicity in cell. Object of this research is to assess changes made in the amount of proline and soluble sugar in response to drought stress made by various PEG densities, in figures of drought tolerant and sensitive cultivars of rapeseed (Slama et al 2008).
MATERIALS AND METHODS

Plant materials and applied drought stress:

Seeds of two canola cultivars were prepared such as SLM 046 & HYOLA 308 as statistics tolerant and drought-sensitive respectively. In order to surface disinfection, seeds were placed into 2.5% sodium hypochlorite (volume/weight) for 20 minutes. After 4-6 times washing them with sterile distilled water, they were placed in 70% ethanol. After forward, several washing with distilled water were done. Then, in order to germination of seeds of statistics tolerant and drought-sensitive they were placed on 2 layers of wet filter papers. After passing 16 days, germinated seeds were moved into medium of aerated Hoagland 1.2 solution. Changing medium was done every 48 hours. After 6 days of seedling establishment in such a situation, PEG 6000 was added to in order to make drought stress in 4 densities as 0, 5%, 10% and 15% to hydroponics substrates. 12-day-old seedlings were affected under drought affect for 2-4 hours. After that, roots and shoots were harvested for measuring the amount of proline and soluble sugars (Dubois et al 1956).

Measurement of proline:

Bates et al method (1973) was used in order to determine amount of proline in root and shoot. Thus for preparing ninhydrin reagent, we pour 1.25 gram of this substance into Erlenmeyer flask then 30ML glacial acetic acid and 20 ml of 6 M phosphoric acid was added. Finally, it was heated gently until ninhydrin solved completely. For measuring proline, firstly 0.2 gram of wet plant sample was measured and was grated in centrifuge machine around 1300 rpm, at 4 ° C for 10 minutes. Then, 2 ml of Filtered extracts were moved into pipes with lid. 2ML ninhydrin reagent and 2 ml acetic acid glacial were added to each pipe. After closing lids of pipes, they were placed in 100 ° C water for one hour. After cooling, 4 ml toluene was added to each pipe. For mixing the 2 solutions, pipes were shaken for 15-20 seconds by using vertex. Finally, we take supernatant phase which is changed into red color containing proline dissolved in toluene. It was placed in spectrophotometer system (GBC CNTR 6 model made in Italy) simultaneously with standard samples. Seed’s numbers were read in wave length of 520 Nm. Protein concentration was determined based on ml/gram of new tissue by using standard curve (Bates et al 1973).

Measurement of soluble sugars:

In order to measure the amount of soluble sugars, 0.2 gram of each of shoots and roots were grated separately in 4 ml of 25 mM sodium phosphate buffer (pH 6.8). Supernatant was used for measuring total sugar. For measuring sample sugar were added 0.5 ml of sugar solution, 0.5 ml of 5% phenol and 2.5 ml of pure sulfuric acid. Immediately after adding sulfuric acid, an exothermic reaction along with orange color appeared that generate too much heat. So it was necessary to cool mixture of the reaction in room temperature for 10 minutes after adding acid. Standard curve was drawn by using various concentrations of sugars as Glucose, Mannose and Rhamnose from 0 to 20 micro grams in ml. Standards absorption along with sample absorption were determined by using spectrophotometer machine in wavelengths as 480 Nm (rhamnose), 485 Nm (Glucose), and 490 Nm (Mannose). Sample sugar amount was determined based on micro gram / gram of wet weight of sample (Zaifnejad et al 1997).

Statistical analysis:

Experiment was done as factorial design based on randomized complete blocks with three replications. The first factor was considered including two varieties and second factor was considered including drought stress in 4 levels. SPSS software was used for analyzing variances of data. Least significant difference (LSD) test in 5% Probability level was used for comparing averages. Drawing diagrams was performed by using Excel software.

RESULTS AND DISCUSSION

Effects of Drought Stress on Density of Proline Amino Acid:

Results showed that drought stress led to increase proline density in 2 two rapeseed variety. This increase among leaf and root of SM046 was more than Hyola 308. In leaf of SLM046, drought stress in all surfaces of it caused significant increase in proline amount. While, in Hyola 308, density increase of proline was meaningful in stress levels of 10% and 15% (figure 1). In root of SLM046 a meaningful increase was seen in density of proline amino acid with decreasing water potential in various levels of PEG. Increasing density of this material rather than control was not significant in Hyola308 in 5% level of stress. Nevertheless, in higher levels of PEG, proline amount were increased significantly (figure b1).

Effects of drought stress on concentration of soluble sugars:

A- Glucose:
Stress of water deficiency led to increase of glucose density in both kinds of canola. This increment in SLM046 was more than Hyola308. In leaf, imposing stress in 5% of PEG caused significant increase in density of glucose in SLM046. But it did not show any significant difference with control. In levels of 10% and 15% stress, glucose density increment was observed in both kinds. This increment was significant in SLM046, but it was not important in Hyola308. In other words, any significant difference was observed between drought levels in glucose density.

In root, stress of water deficit caused increment of concentration of glucose in rapeseed. The amount of sugar concentration amount in SLM046 was more than Hyola308. A significant rise in glucose concentration was seen by decreasing water potential in 5%, 10% and 15% of PEG levels at both of them.

**B- Mannose:**

In Rapeseed leaves under study, the imposed stress led to increasing mannose concentration. Of course, amount of increment was more in SLM046. Stresses of 5% and 10% of PEG led to significant growth in mannose concentration. But 15% stress level led to decreasing mannose concentration. By the numbers, it did not show any significant difference with 10% stress. In Hyola 308 figure, 10% stress led to significant increment of mannose. 10% and 15% stresses did not show significant difference in numbers.

**Fig. 1:** Effects of water stress applied using different concentrations PEG6000 the leaf proline (a) (LSD0.05=0.33) and root (b) (LSD0.05=0.01) the two varieties SLM046 and hyola308. Each column represents the mean of three replicates as S.E.
Fig. 1: Effects of water stress applied using different concentrations PEG 6000 the glucose concentration in the leaves (LSD0.05=0.88) (a) and root (LSD0.05=0.25) (b) the two varieties SLM046 and hyola308 each column represents the mean of three replicates as S.E.

Fig. 2: Effects of water stress applied using different concentrations PEG6000 the concentration of mannose leaves (LSD0.05=0.63) (a) and root (LSD0.05=0.41) (b) the two varieties SLM046 and Hyola 308 each column represents the mean of three replicates as S.E.
Water deficit in root caused increasing concentration of mannose sugar in SLM046 more than Hyola 308. In SLM046 and Hyola 308, reducing water potential in all levels of PEG caused increment of concentration of root mannose. The increment in SLM046 has been significant in all levels. But significant difference was not seen between 15% and 10% stresses.

C- Rhamnose:

Drought stress caused increasing concentrations of rhamnose in two cultivars of rapeseed in leaves and roots of rapeseed. But increment in SLM046 was more. In leaf of SLM046 and Hyola308, the most concentration of rhamnose was seen in 10% stress of PEG which did not show significant difference with drought caused by 15% of PEG. In roots of the two tested rapeseed cultivars, stresses of 5% and 10% of PEG caused significant increment in concentration of sugar of rhamnose. According to the obtained results in this research, we observed that drought stress causes increased proline and soluble sugars in two cultivars rapeseed. These results showed that producing these osmotic regulators is a typical response to drought stress situation. Increased concentration of proline is a kind of compatibility for overcome stress in plants under stress. Proline under stress conditions can have various operations such as causing osmotic balance, preservation of protein structure and cell membrane, stabilization of intracellular structures and removing free radicals (Van Den Berg and Zeng, 2006). After stress, these amino acids and carbohydrate are broken down easily. In the test, always proline concentration in SLM046 has been more than Hyola308. There by, SLM046 type showed better drought resistance than other types (Sinaiki et al 2007). Concentration Increase of proline has been reported under drought stress condition in Sorghum, Wheat, Sunflower, Corn and Rapeseed too. In these plants, proline amount has been in more in resistant type. But in potato, prolin amount in resistant type has been decreased. This is along with this hypothesis that proline is not a feature of adaption to stress but it is a sign of stress (Xu et al 2008). Many stressful environmental conditions affect carbohydrate metabolism and growing assimilates. Increasing amount of soluble sugars has been reported under conditions of salinity, water logging and freezing. In drought stress condition; osmotic regulators could increase power absorbing water from the soil by root (Mostajeran and Rahimi-Eichi, 2009). In another study on two types of corn, the concentration of soluble sugars was increased by imposing drought stress. In addition, in studies on rice, wheat and other types of rapeseed, imposing drought stress was led to increasing the amount of sugar of resistant type contrast to sensitive type (Manivannan et al 2008). In the test, SLM046 type produced higher numbers of soluble sugars under various levels of stress. Therefore, this type is able to absorb more amount of water in drought stress condition by increasing osmosis potential. Generally, increment of proline concentration and soluble sugars in leaves and roots of SLM046 type was more than Hyola 308. So it seems that accumulation rate these osmotic regulators are related to drought resistance in SLM046 type in this research.

REFERENCES


