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Effect of UV-C Radiation on Drought Tolerance of Alfalfa (*Medicago sativa*) Callus

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Abstract: Water stress and unfavorable climate decrease the growth and development of plant globally. *Medicago* is a valuable plant as a source of food for animal, foliage and for medicine. Since, Iran is located in a dry area, consequently, the study of plant responses to water stress is important. In this study UV-C radiation was used to induce physiological and genetic changes in alfalfa callus. *In vitro* grown calluses of *M. sativa* were exposed to UV-C for 0,15, 30 and 60 min and then, were transferred to MS medium containing 0, 2, 5, 10, 20 and 30% PEG. All explants were kept in the dark in the culture room. Calluses tolerate to osmotic stress were selected according to fresh weight. Results showed that radiation of UV-C for 60 min increased the osmotic tolerance and decreased the effect of drought stress.

Key words: Callus, *Medicago sativa,* UV-C, PEG, drought stress

INTRODUCTION

Rising human and animal populations and their ever expanding needs for food, fodder and feed have exerted tremendous pressure on agroecosystems. The continued increase in food production in order to keep pace with the unabated population growth in the world is a constant worry for scientists and planners. Four-tenths of the world's agricultural land lies in arid or semi-arid regions, consequently salinity and drought are the two major environmental stresses that limit plant growth and productivity[1]. Other agricultural regions have consistently low rain-fall and rely on irrigation to maintain yields. In both circumstances, crop plants which can make the most efficient use of water and maintain acceptable yields will be at an advantage. To access this approach, understanding cellular adaptation mechanisms to drought stress and its biological process that protects organisms against the lethal effects of dehydration may leading to cope with this global problem.

Medicago sativa is a leguminous plant species that originated in Asia and Iran. This plant species has been grown for a variety of purposes such as soil improvement, animal feed, medicinal uses and a suitable foliage[2]. Despite extensive studies on NaCl tolerance of leguminous plants, very few studies have been conducted on *M. sativa* explaining drought tolerance.

Genetic improvement of drought tolerance has traditionally been a problematic topic in plant breeding for a variety of reasons, among which is the lack of clearly defined selection criteria for tolerance. Moreover, plant breeding using conventional procedure is time consuming and sometime impossible for a number of plant species[3]. New technology such as plant cell and tissue culture, gene transformation and mutation technology are possible solutions to improve the productivity of modern agroecosystems and drought tolerance in plants[4].

The most common way to obtain and select variant clones or mutagens is to expose disorganized cells or callus to specific condition which allow for the survival of only a small fraction of the population presumptively consisting of spontaneous mutants or physiological changes adapted to new condition. These conditions include high concentration of metabolites, salt, PEG, toxic drugs and environmental stress[5]. *In vitro* selection technology combined with spontaneous or active mutagenesis has been effective in altering or isolating genetic variability for desirable characters. For example, it has already been reported that up to 10 fold increase in the frequency of 5-methantryptophane resistant carrot cells increases up to 10 fold in the presence of UV light[6]. UV radiation can affect many aspects of plant processes at the physiological and DNA level depending on wave length (UV-C: below 280 nm, UV-B: 280-320 nm and UV-A: 320-390 nm)[7].

This study was aimed to investigate the possibility of UV-C response in *Medicago* calluses for selection to osmotic stress using Poly Ethylene Glycol (PEG)[8].

MATERIALS AND METHODS

Seeds of *Medicago sativa* cv. Rehnani were obtained from Natural Resource of Isfahan. Iran. Seeds were surface sterilized in ethanol (70%) for 30 sec then in

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sodium hypochloride (20%) for 20 min followed by 3-4 times washes with sterile distilled water under aseptic condition. Seeds were then transferred on MS medium[9]. After 30 days calluses were initiated from stem segments of *in vitro* grown plants on MS medium containing kinetin, 2,4-D and NAA (2 mg each). Propagated calluses were then transferred to the same medium supplemented with 0, 2, 5,10, 20 and 30% PEG (MV 6000) as osmotic stress (PEG was added to MS medium according to diffusion based method of Girma and Kreig[10]. Final osmolarity of the medium with PEG were 217, 230, 270, 321, 720 and 1320 mmol kg^{-1}, respectively). Calluses in the culture containers were then exposed to UV-C for 15, 30 and 60 min from 15 cm distance of UV source (approx. I.5 kj m^{-2}). calluses unexposed to UV-C were used as control. All experiments were carried out in a Factorial Design with 10 replication and 5 pieces of callus per each replicate. After UV radiation all calluses were kept in the dark in 25°C culture room. The osmotic tolerant calluses were selected according to the fresh weight after 18 days. For assessment of acid phosphatase activity, at the end of day 18th, 1 g of callus from differently treated explants was ground with a mortar and pestle in 1 mL extraction buffer and an enzyme assay was conducted according to Julie *et al.*[11] for three replicates.

RESULTS AND DISCUSSION

Figure 1a-d show a few important points: 1) as a general phenomenon increasing of PEG concentration in the culture medium resulted in a decrease in fresh weight of callus. 2) Radiation of UV-C on calluses changed the pattern of growth in the PEG treated callus. For instance, radiation of UV for 15 min increased fresh weight up to 10% in the medium containing 0 and 20% PEG. When calluses exposed to UV light for 30 min the callus growth increased 17.37%. In this condition UV increased the fresh weight of callus in medium with 0, 2, 20 and 30% PEG. Combination of osmotic stress and UV radiation for 60 min increased fresh weight of calluses up to 37.96%. Under the high level of osmotic stress (medium with 10% PEG) fresh weight was increased up to 50%. In comparison with calluses unexposed to UV, those radiated showed better cell division and growth (Table 1).

Increase of PEG concentration resulted in increased acid phosphatase activity of calluses. The difference between callus growth treated with 0, 2, 5, 10 and 20% PEG was not significant but, significant differences were observed between 30% and other concentration of PEG. While 60 min radiation of UV decreased the enzyme activity (Fig. 2h), 15 and 30 min radiation had no

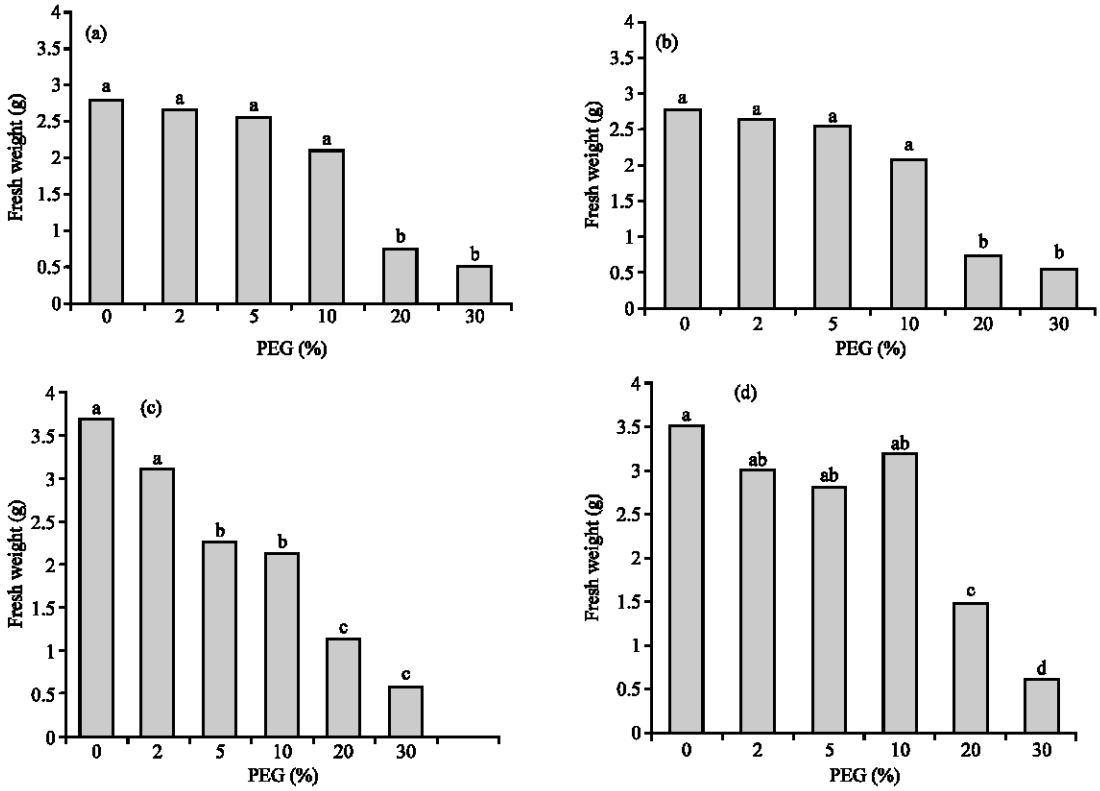

Fig. 1: Effect of UV-C radiation on fresh weight of *Medicago* callus treated with PEG. a: no radiation, b, c, d: radiation for 15, 30 and 60 min (uncommon letter are significant $p<0.05$)

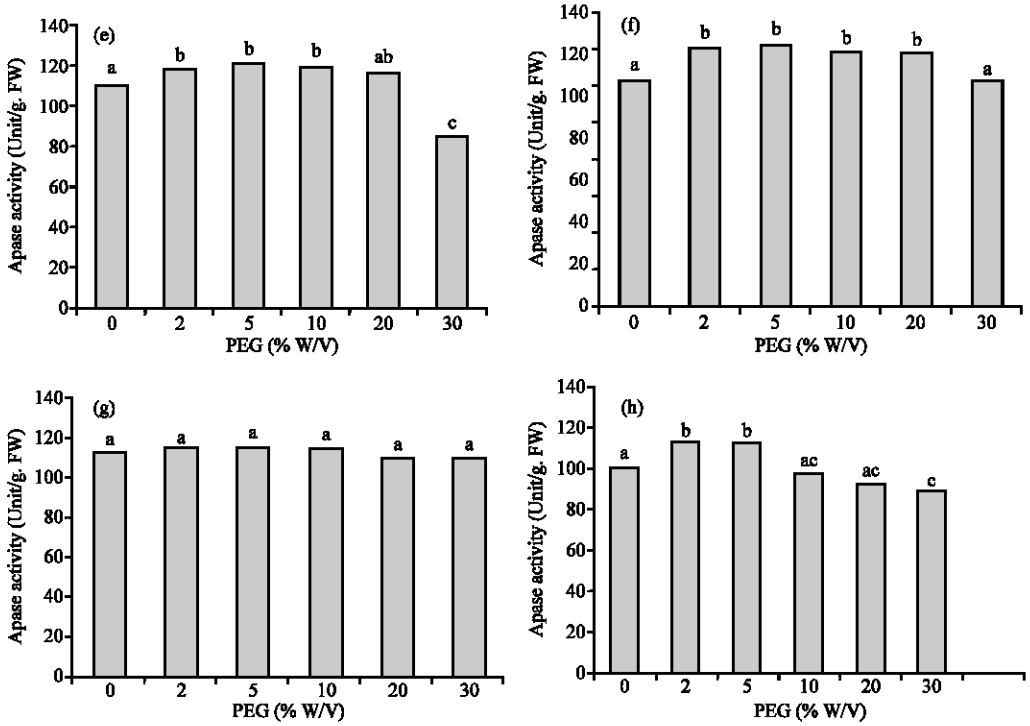

Fig. 2: Effect of UV-C radiation on acid phosphatase activity of callus under PEG treatment. e: no radiation, f, g, h, radiation for 15, 30 and 60 min

Table 1: Relative growth of callus (g) treated and untreated with UV-C under osmotic stress in compare with control

	PEG (%)					
UV-C (min)	0	2	5	10	20	30
15	+19.56	+0.75	-16	0	+10	-20
30	+32.24	+15.41	-12	+1.44	+57.14	+10
60	+26.81	+12.78	+9.44	+52.17	+108.57	+18

(+): increasing and (-): decreasing of growth

significant effect on Apase activity in calluses (Fig. 2f and g). However compared with unexposed callus (Fig. 2e) cultured in medium with 30% PEG, radiated calluses for 60 min showed higher of Apase activity (20.29%).

Plants have evolved a different mechanisms for adaptation to osmotic stress. One of these is osmoregulation. This may occur in callus grown under PEG treatment. For example glycine betaine and proline found in scores of higher plants behave as osmoprotectants. Many investigators proposed that the molecules accumulate in plant cells during osmotic stress and prevent damage from cellular dehydration by balancing the osmotic strength of the cytoplasm with that of the environment[4]. A similar mechanism may exist for drought tolerance in *Medicago sativa* callus.

The effect of UV-C on plant cells (e.g. protoplast and cell suspension) has already been tested in context of DNA mutagenesis in *Arabidopsis thaliana* with increasing UV-C does[5]. The drought tolerance of callus can be explained by several characteristics of plant cells. These include, 1) the presence of provitamin D, this molecule has been shown to act as a natural sunscreen from high energy UV radiation, 2) the presence of flavonoids, since it has been shown that flavonoids protect DNA from damage, 3) the presence of cell walls that reflect up to 99% of UV radiation. In present experiments UV-C induced antocynin production on the surface of calluses but whether or not it is a source of UV protection remained to be studied[12].

Solid media supplemented with PEG have been frequently used for tissue culture experiments. Lowering water potential of the medium decreases the cell division and growth of callus. Sometime this condition enhances regeneration. For example, in the process of plant regeneration from *Medicago sativa* callus, root and shoot are formed in medium containing mannitol (data are not shown). Similar results were reported in *A. thaliana* under water stress condition.

In present experiments callus growth decreased with increasing the concentration of PEG. When callus exposed to UV-C the effect of drought stress on growth was lower than unexposed calluses. This could be due to genetic changes (somaclonal variation) or epigenetic changes (physiological changes)[13].

Important features of Apase can be scored for enhance phosphatase activities and expression to reutilize Pi from stored reserve during germination, growth or senescence and to recycle or scavenge Pi under drought stress condition[14]. Increasing Apase activity of calluses exposed to UV-C and PEG stress may act by maintaining a certain level of Pi which can be co-transported with H+ along a gradient of proton motive force[15].

However, the relationship between Pi utilization and UV-C radiation at molecular level need to be studied in details in the future.

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