



RESEARCH PAPER

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The effects of priming by folic acid and hydrogen peroxide on morpho-physiological traits in cannabis seeds (*Cannabis sativa* L.)Shirin Karbalaye Golizadeh¹, Tooraj Mir Mahmoodi^{*2}, Nabi Khaliliaqdam³¹*Department of Agronomy and Plant Breeding, Mahabad Branch, Islamic Azad University, Mahabad, Iran*²*Department of Agronomy and Plant Breeding, Mahabad Branch, Islamic Azad University, Mahabad, Iran*³*Department of Agriculture, Payame Noor University, Saghez, Iran***Key words:** RWC, Spad, Seed vigor index, stomata conductance, specific leaf area.

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Abstract

Effect of various folic acid and hydrogen peroxide concentration on some traits of cannabis performed based on randomized complete blocks design in research green house of Islamic Azad University of Mahabad, Iran. Experiments treatments were levels of folic acid (0,5,10,15,20,25,27Mm), hydrogen peroxide (0,7.5,15,22.5,30,37.5,40 Mm) and control (non-primed) which replicated 4 in 24 and 6 h in 2014. Laboratory parameters measured included, seed vigor index, germination rate and seedling dry weight And measuring the greenhouse includes RWC, Stomata conductance, dry weight root and plant, specific leaf area and spad. Folic acid was positive trend than hydrogen peroxide. compared to the control and primed with distilled water for 6 hours, respectively about 16 and 11 percent were biological function.

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Introduction

Cannabis is a dioeciousness plant, and a year of orders utricles and Family Cannabinaceae (*Cannabis sativa* L.). The plant leaves are serrated claws with five to seven leaflets (Yoshimatsu *et al.*, 2004). Cannabis for various aspects of human attention. Cannabis is one of the best sources of natural fiber. Cannabis seed oil contains essential fatty acids for human nutrition cannot replace fish oil. the medicinal use of cannabis Has a long history (Pinarkara *et al.*, 2004; Hazekamp, 2009).

Priming is a procedure that partially hydrates seed, followed by drying of seed, so that germination processes begin, but radicle emergence does not occur. A method to improve the rate and uniformity of germination is the priming or physiological advancement of the seed lot (Finch-Savage, 2004; Halmer, 2004). Seed priming is the soaking of seeds in a solution of any priming agent followed by drying of seeds that initiates germination related processes without radicle emergence (McDonald, 1999).

The effects of seed priming are associated with the repairing and building-up of nucleic acids, increased synthesis of proteins as well as repairing of the membranes (McDonald, 2000). Priming also enhances the activities of anti-oxidative enzymes in treated seeds (McDonald, 1999; Wang *et al.*, 2003; Hsu *et al.*, 2003). Seed priming has been used to improve germination, seedling emergence and yield (Khan, 1992; Ghassemi-Golezani *et al.*, 2008; Ghassemi-Golezani *et al.*, 2010). The beneficial effects of priming have been demonstrated for many field crops such as maize (Parera and Cantliffe, 1994), sunflower (Singh, 1995), sugar beet (Sadeghian and Yavari, 2004), barley (Abdulrahmani *et al.*, 2007), lentil (Ghassemi-Golezani *et al.*, 2008a), chickpea (Ghassemi-Golezani *et al.*, 2008b), pinto bean (Ghassemi-Golezani *et al.*, 2010a) and winter rapeseed (Ghassemi-Golezani *et al.*, 2010). pre-treatment seed with hydrogen peroxide increases germination percentage shoot emergence, shoot and

root length and weigh more (Amjad *et al.*, 2004; Cavusoglu and Kabar., 2010). Therefore, given the importance of folic acid and hydrogen peroxide on growth rate and seedling establishment in the present study the use these materials on morpho-physiological traits of cannabis was tested.

Materials and methods

Laboratory Experiment

cannabis seeds (*Cannabis sativa* L.) were abstained from the Pakanbazar in Esfahan. Laboratory tests were carried out at the seed Technology Laboratory of Mahabad Branch of Islamic Azad University, Iran, using randomized completely (CRD) design with 4 replicates in 2014. Treatments considered as the control (non-primed) and priming with folic acid in (0.5, 10, 15, 20, 25, 27 Mm) and hydrogen peroxide (0.7.5, 15, 22.5, 30, 37.5, 40 Mm). for priming, cannabis seeds soaked in various concentration of folic acid for 24 hours and hydrogen peroxide for 6 hours. After priming, sample of seeds were remove and 30 seeds in petri dishes with a diameter of 100 mm between two layers of filter paper that had been soaked with distilled water and germination in germinator a $25\pm 2^{\circ}\text{C}$ with a relative humidity of 42 percentage were taken.

Green house Experiment

The experiments green house were carried out based on randomized complete blocks (RCB) design with 4 replicates in 2014 at the research green house of the Islamic Azad University of Mahabad. Temperature greenhouse $33\pm 3^{\circ}\text{C}$, night Temperature $14\pm 3^{\circ}\text{C}$ and moisture content of about 80-25 percent now. The experiment, before potting soil used in the laboratory analysis of the soil (Table 1). Soil in plastic pots 16 Cm in height and 15Cm in diameter and primed seeds were sown at a depth of 1.5 cm. And once a week until the 4- leaf stage in each pot was given 300Cc of water and traits such as stomata conductance, Phenol, Chlorophyll index, specific leaf weight were measured.

Table 1. Selected properties of soil at the experimental site.

SOV	PH	P(ppm)	K(ppm)	Soil texture(%)	Saturation percent(%)	Clay(%)	Sand(%)	Silt(%)	Organic carbon(%)
	7.94	13.42	6.7	Loam	37	22	38	40	1.53

Statistical Analysis

Data was analyzed using SAS software and statistical averages, with Duncan test at the 1% and 5% level were graphs were plotted using Excel software.

Result and discussion

Germination Rate

Analysis of variance showed germination rate cannabis seed was affected by folic acid and hydrogen peroxide (Table2).The comparison showed that

priming with various concentration of folic acid had a positive effect on the speed of germination of cannabis seeds so to the level of 15mm highest germination rate was observed in the first 12 hours this the decline found in higher concentrations where the highest concentration of primed with folic acid (27mm) folic acid is approximately 20% compared to 15mm decreased the priming process with hydrogen peroxide in the first 12 hours, and have a negative effect on germination rate was reduced (fig1).

Table 2. Effect of priming hydrogen peroxide and folic acid in treat laboratory on cannabis sativa.

S.V	DF	Mean Square				
		Germination Rate 1	Seedling dry weight	Seed vigor index1	Germination Rate 2	Seed vigor index2
Repeat	3	0.00002 ^{ns}	0.00002 ^{ns}	0.0000006 ^{ns}	0.00001 ^{ns}	0.0000005 ^{ns}
Folic acid	7	0.0002 ^{**}	0.003 ^{**}	0.000005 ^{**}	0.000303 ^{**}	0.00001 ^{**}
Error	21	0.00001	0.00003	0.0000003	0.000012	0.0000003
cv (%)		8.25	5.37	12.12	6.2	10.2

S.V	DF	Mean Square				
		Germination Rate 1	Seedling dry weight	Seed vigor index1	Germination Rate 2	Seed vigor index2
Repeat	3	0.000039 [*]	0.00012 ^{ns}	0.00000136 ^c	0.00024 [*]	0.00000008 ^{ns}
H ₂ O ₂	7	0.00006 ^{**}	0.00069 ^{**}	0.0000039 ^{**}	0.0002 [*]	0.00001 ^{**}
Error	21	0.00001	0.00006	0.0000004	0.00005	0.0000009
cv (%)		4.76	8.6	7.87	8.86	10.4

*,** and NS indicate significant difference at 0.05, 0.01 and not significant respectively, S.V= Source of variation; DF = Degree of Freedom; Seed vigor index 1= first 12 hours; Seed vigor index2= second 12 hours; Germination 1=first 12 hours; Germinating 2= second 12 hours.

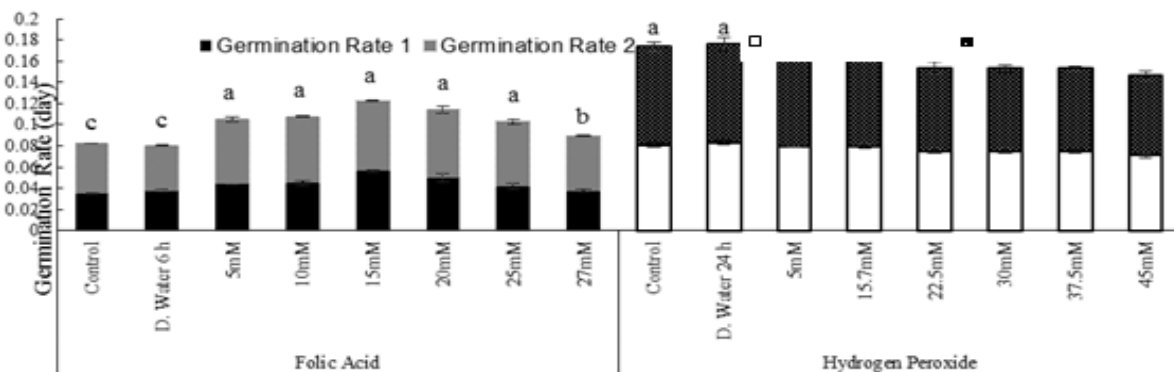


Fig. 1. Effect of priming hydrogen peroxide (H₂O₂), folic acid (B9) and distilled water (D. water) on germination rate.

Seedling dry weight

Seedling dry weight was significantly influenced by priming was with folic acid ($P>0.01$) (Table 2). Accordingly, cannabis seed priming with folic acid was increased seedling dry weight. Even at a concentration of 5 mm to 0/13 gr seedling dry weight compared to control, about 57 percent increased. Also changes with increasing concentration of folic acid as

well as other characteristics of satiety deteriorate. Changes in dry weight of seedling in seed priming experiments with hydrogen peroxide was significant ($P>0.01$). Maximum dry weight of the cannabis plant 0/1 gr was treated with 15.7 mm of hydrogen peroxide, But at higher concentrations adversely was affect seedling dry weight (fig2).

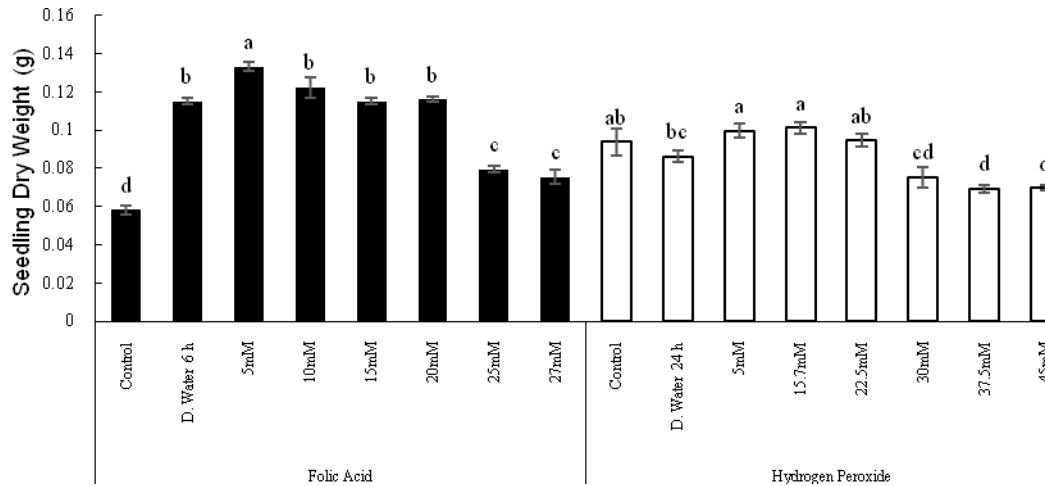


Fig. 2. Effect of priming hydrogen peroxide(H₂O₂), folic acid (B₉) and distilled water (D.water) on seedling dry weight.

Seed Vigor Index

Analysis of variance showed that seed vigor index in the first 12 hours was significantly influenced by priming technique was at a with folic acid (Table 2). Based on this trend, as the speed of germination and seedling dry weight up to 20 mm folic acid has been positive and continued to increase to 25 and 27mm has trend and seed vigor index decreased 32 percent compared to baseline concentration (5mm) was associated with folic acid. The results showed that priming with hydrogen peroxide reduced seed vigor index in the first and second hours, therefore, a negative reaction on the concentration of hydrogen peroxide on seed vigor index (fig3).

deployment on seed primed (murugu *et al.*, 2004). Accelerate the increased activity of enzyme alpha-amylase, increased levels of bio-energy charge in the form of increased amounts of ATP, increased synthesis RNA and DNA, increase the number and improve the function is mitochondria(Afzal *et al.*, 2002). The primed seed germination of some metabolic and biochemical benefit will be realized. For example, the seeds of proteins and carbohydrates the effect enzyme and hydrolysis reactions of the break and are ready to participate in the process of germination. Also, the function and structure of cell membranes compared to seed primed seeds can be seen in a more favorable position. This issue can be investigated by studying the electrical conductivity of seed extract so that leakage of intracellular metabolites of primed seed was lower membrane and consequently the electrical conductivity of the seed extract is less. This is primed of sweet corn, sugar

Reports indicate much improved germination behavior and it is related indicators such as average time of germination, seed vigor index, Root length, length Plumule, The seed germination rate and initial

beet, plum, radish, wheat and barley has been proven. It can also be justification for better germination of seeds is treated (pil and Nicker, 2001). The present in

experiment seeds primed with folic acid is positive results in increasing the indicators along mentioned.

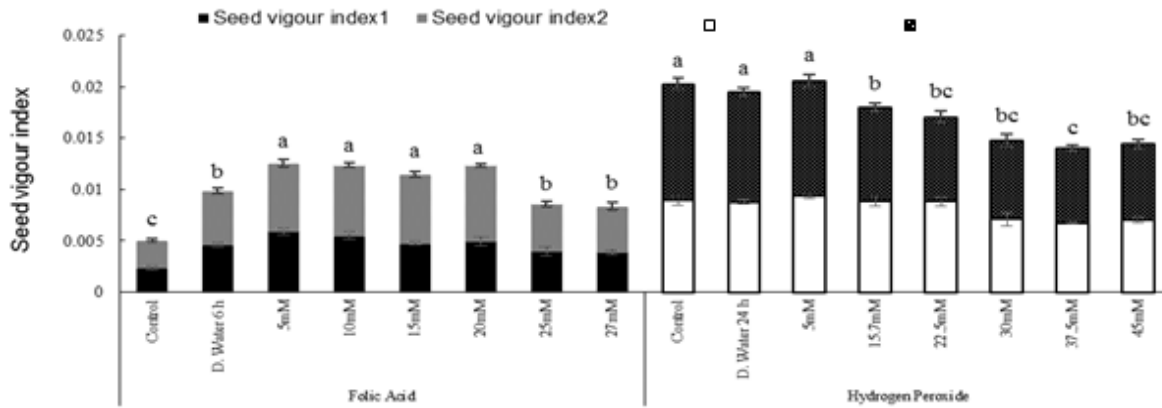


Fig. 3. Effect of priming hydrogen peroxide(H₂O₂), folic acid (B9) and distilled water (D.water) on seed vigor index.

Stomata conductance

The results (Table3) indicate a significant priming effect of folic acid ($P>0.0$) was on stomata conductance. Accordingly, cannabis seed priming with folic acid compared to control (non-prime) at different levels a positive trend showed. But the comparison revealed cannabis seeds primed with folic

acid at a concentration of 15mM better performance 405.56 mM on stomata conductance compared to the control and primed with distilled water for 6 hours, respectively, 11 and 16 percent have shown. The hydrogen peroxide has the opposite trend with increasing concentration of control is primed (fig 4).

Table 3. Effect of folic acid and hydrogen peroxide on treat morpho-physiologic on cannabis in green house.

S.V	DF	Mean Square				
		RWC	Stomata Conductance ₁	Specific Leave Area	SPAD	Plant Dry Weight
Repeat	3	7.54 ^{ns}	1841.4 ^{ns}	3532.39 ^{ns}	6.461 ^{ns}	19.198*
Folic acid	7	210.32**	11023.2**	4273.92*	18.008*	21.734**
Error	21	15.6	1440.35	1357	6.322	5.53
cv (%)		19.6	10.9	16.23	12.24	12.36

S.V	DF	Mean Square				
		RWC	Stomata Conductance ₁	Specific Leave Area	SPAD	Plant Dry Weight
Repeat	3	10.25*	1721.98 ^{ns}	246.15 ^{ns}	9.0537 ^{ns}	30.56 ^{ns}
H ₂ O ₂	7	193.18**	2277.54*	1785.67 ^{ns}	12.81403*	5.556 ^{ns}
Error	21	2.942	730.1	831.13	5.0319	12.93
cv (%)		12.2	10.28	13.67	12.48	20.5

*,** and NS indicate significant difference at 0.05, 0.01 and not significant respectively, S.V= Source of variation; DF = Degree of Freedom.

Acid pivotal role in the regulation of physiological processes such as seed germination, stomata conductance, inhibition of ethylene biosynthesis and

chlorophyll content, fruit production, heat production and plays glycolysis (El-Tayeb 2005; Popova *et al.*, 2003).

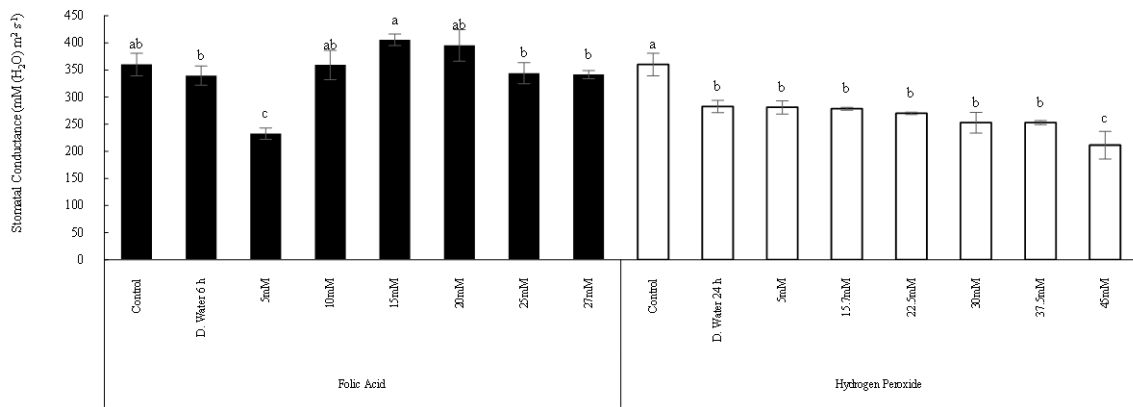


Fig. 4. Effect priming of folic acid (B9),Hydrogen peroxide(H₂O₂) and distilled water (D.Water) on stomatal conductance.

Relative water content (RWC)

The results (Table 3) indicate effect a significant on the relative water content was primed folic acid and hydrogen peroxide ($P>0.01$). Accordingly, the highest relative water content 29.5 percent was of priming with folic acid at a concentration of 15 Mm to 6 hours than control about 45 percent compared to distilled water for 6 hours, showed increase a 6.5% but decreased at higher levels. While this is not a desirable outcome primed with

hydrogen peroxide. However, as the concentration of folic acid in a better outcome. The highest relative water content in priming experiments with hydrogen peroxide, distilled water for 24 hours at a rate is of 21.6 percent. That statistically the priming concentration of 5 and 15.7 mmol of hydrogen peroxide are not different significantly and placed in a statistical. Accordingly, the lowest relative water content of the treated is hydrogen peroxide in 45 and 37.5 mm concentration (fig 5).

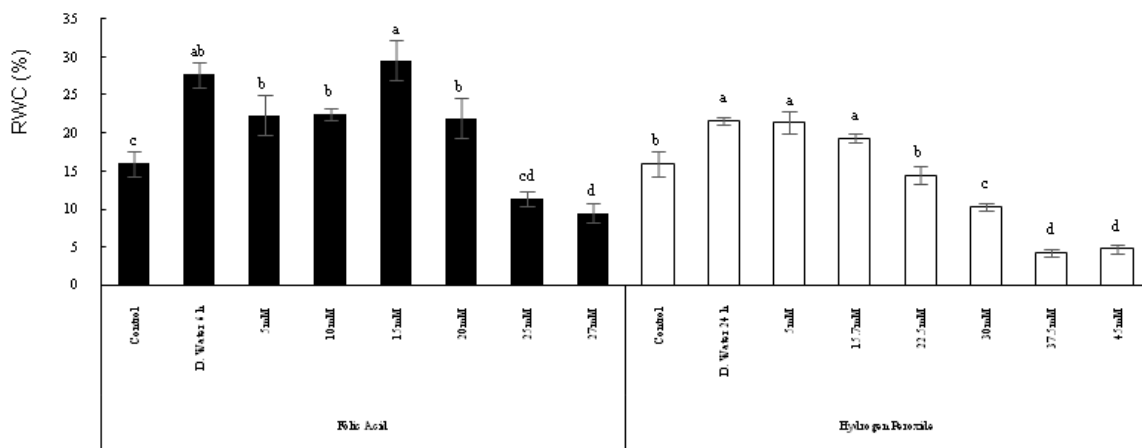


Fig. 5. Effect of priming hydrogen peroxide(H₂O₂),folic acid (B9) and distilled water(D.water) on RWC.

Water potential physiological direct relation to the amount of evapotranspiration that through the cuticle and stomata and photosynthetic capacity and also have a look at the amount of water absorbed by the root. (Antholin *et al.*, 1993) reported the

accumulation of soluble sugars react quickly to changes in the relative water content (RWC) and is leaf potential water, stomata controlling of loss water, as an early event in plant response to stress is the lack of water content leading to limit the carbon

absorption by leaves (cornic and Massacci, 1995). Evidence suggests that stomata conductance is increased stomata (Sajjadinia *et al.*, 2010) thus reduce resistance photosynthesis and impact performance (Atteya, 2003). So better seedling establishment and initial power up due to higher leaf area, leading to increased efficiency of water use in the early growth period. Efficient use of water from the soil in the early stages of growth, biomass and yield will follow increases (Lopez *et al.*, 1995, 1996).

Chlorophyll index (Spad)

Analysis of variance showed (Table 3) chlorophyll index was significantly influenced by the primed seeds was water and folic acid ($P>0.01$). Accordingly, the maximum chlorophyll index for primed treated with distilled water for 6 hours which had increased by about 24 percent in addition to seeds primed with distilled water for 24 hours increased that can be said other than prime time prime sources in the use of these techniques is important (fig 6).

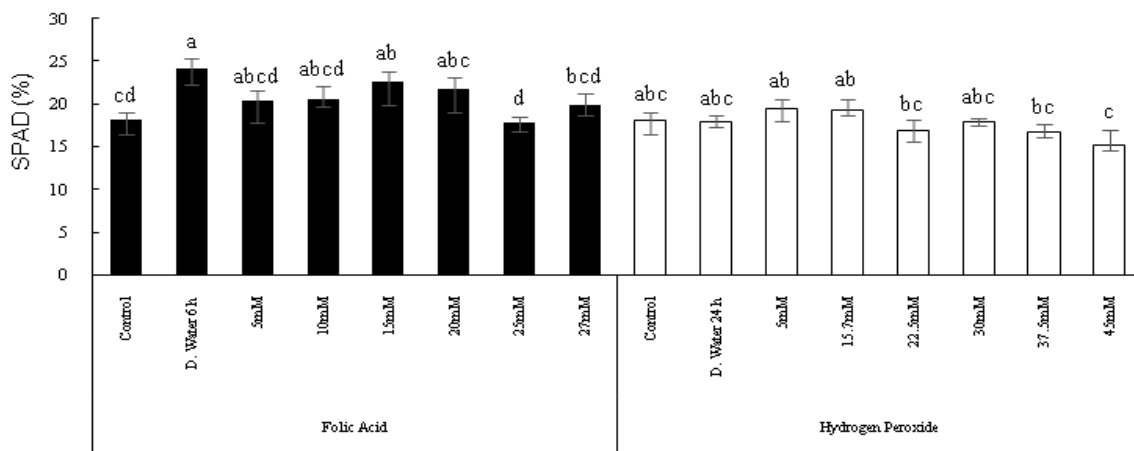


Fig. 6. Effect of priming hydrogen peroxide(H₂O₂), folic acid(B₉) and distilled water (D.Water) on chlorophyll index(spada).

Specific leaf Area

Leaf area directly related to chlorophyll content, stomata conductance, leaf water potential and photosynthetic capacity is ultimately influenced by different concentration was primed of folic acid (Table 3). Accordingly, by increasing the

concentration of folic acid primed with respect to levels (5mm) decreased leaf area where the prime concentration of 27 mm folic acid thus the reference concentration (5mm) was associated with a reduction of 37 percent (fig 7).

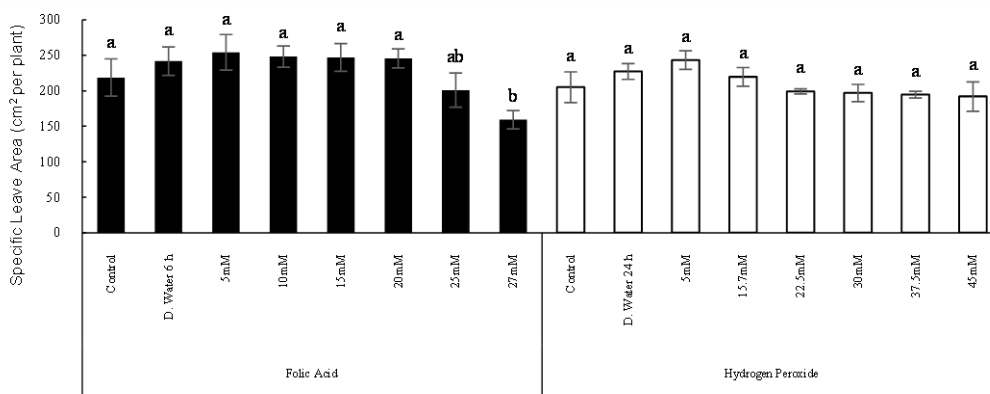


Fig. 7. Effect of priming hydrogen peroxide (H₂O₂), folic acid (B₉) and distilled water (D.water) on Specific Leaf area.

The amount of light energy in plants and the consolidation of the physiological parameters that determine the growth and yield. Seed priming can in plants with increased chlorophyll content and photosynthetic pigments, the rate of photosynthesis increase and by the power source and provides simulate increase and improve performance into taken (Roy and Srivastava, 2000).

Dry weight plant and Root

Plant dry weight ($P>0.01$) as biological yield was influenced by priming with folic acid (Table 3). Dry weight of plant physiological conditions affect both

the absorption of minerals, photosynthetic capacity, plant water status and the amount of storage carbohydrates and sugar is dissolved. Due to the increase in leaf area and photosynthetic capacity factors include the relative water content, stomata conductance and chlorophyll index (spad) with prime folic acid compared to control increased plant dry weight relative the maximum amount of dry weight of the treated base was concentration (5mm). Compared to the control and primed with distilled water for 6 hours, respectively about 16 and 11 percent were biological function (fig 8).

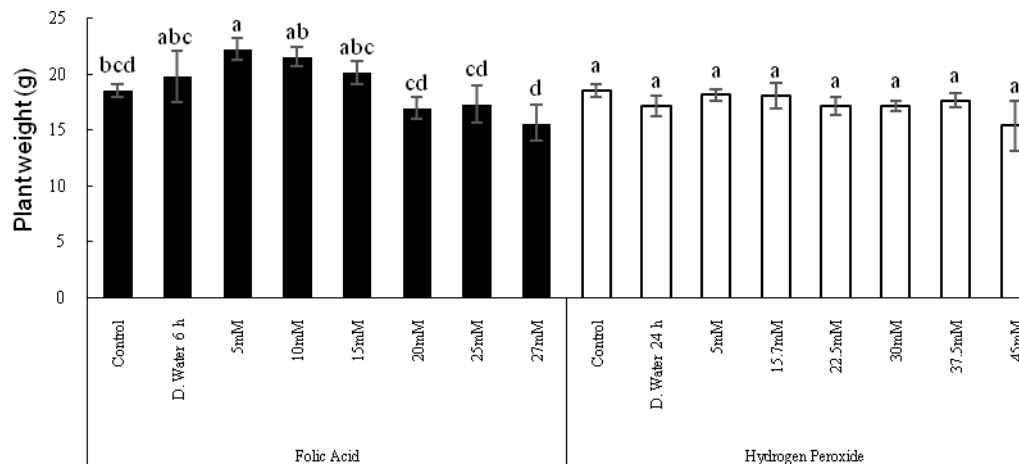


Fig. 8. Effect of priming hydrogen peroxid (H₂O₂), folic acid (B9) and distilled water (D.water) an dry weight plant and root.

Biological yield (dry matter) and plant fresh weight is influenced by various factors such as genetics, harvest time, absorption of minerals, environmental condition etc. are located. According to the performance effect of folic acid on germination and seedling establishment of improved of nutrients absorption and increased photosynthetic capacity and ultimately an important role in determining the final density of there is the unit level.

Conclusion

In general, the efficiency and the importance of different sources of primed seeds primed surface also plays an important role in the efficiency of this

technique. And this study result showed the folic acid causes better seedling establishment and increase the efficiency of photosynthetic capacity plant has a direct impact on biological performance.

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