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Effects of Seed Priming with Gibberellic, Salicylic Acids and Mycorrhizal Inoculation on Lentil (*Lens culinaris* L.) Yield and its Components

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ABSTRACT

A field experiment was conducted to study the effect of mycorrhizal inoculation and seed priming with plant growth regulators on yield and yield components of lentil as factorial base on the randomized complete block design in four replications at the research farm of Gonbad Kavous University of Iran during 2013-2014. First factor included application of the mycorrhiza at three levels (control, inoculated soil using *Glomus intraradices* and *Glomus mosseae*) and seed priming at five levels (hydro-priming with distilled water, seed priming with GA3 100 ppm, priming with SA 100 ppm, priming with GA3 100 ppm + SA 100 ppm and non-primed seed as control was the second factor. On the basis of results, days to 50% flowering was significantly decreased with the application of mycorrhizal inoculation along whole treatments of priming except *G. mosseae* in combination with both hormones level. It was also observed that combined treatments of *Glomus* intraradical inoculation with hydro priming caused most early flowering, the number of pods in the plant, ratio of the filled pod, grain weight of lentil eventually higher yields and harvest index of lentil over other treatments. Thus mycorrhizal inoculation can help to minimize synthetic herbicides and develop biofertilizer in sustainable agriculture.

Key words: Combination treatments, Harvest index, Hydro priming, Pod number.

INTRODUCTION

Lentil is an important cool season food legume of our nation and positions next to chickpea (Dixit *et al*., 2017). Lentil reportedly contains 24-28% protein, 30-40% minerals and 22% vitamins in dry seeds. Mean yield of lentil is 1077 kg/ha in the world and 560 kg/ha in Iran (FAO, 2016). Due to its ability in nitrogen fixation, lentil helps to maintain soil fertility and finally yield of other crops will be increased in rotations (Parsa and Bagheri, 2008). However, poor yield can result from poor germination, weak seedling emergence, weak seedling, generally undesirable plant establishment. This situation has forced us to look for ways to overcome early plant establishment problems. One of the method for increasing germination and seedling quality is seed priming. Priming is a process in which seeds absorb water and initiate the seed germination process prior to planting. Germination percentage is important for crop establishment yield. Priming is a simple and effective technique to suffer various environmental stresses and can improve germination percentage. Seed priming is normally practiced with water and can be improved further by selection of inorganic chemicals and growth regulators. Seed priming with growth regulators may improve the physiological efficiency and may play a significant role in raising the productivity of the crop. Normally lentils have large number of flowers but most of them drop resulting in poor yield. Available literatures suggest that growth regulators can be used as potential tools to enhance the yield of pulses (the dried edible seeds of certain plants in the legume family) by minimizing the several physiological constraints (Singh, *et al*., 2017). Some phyto-hormones such as salicylic acid and gibberellic acid can be used in priming (Zaki and Radwan 2011).

Pretreatment of salicylic acid depending on the desired concentration, species and environmental conditions during growth play an important role in regulating various physiological processes such as seed germination, plant development and photosynthesis (Iqbal and Ashraf, 2006). Gibberellic acid is involved in many physiological processes such as cell division, increase in cell length (Khoshkhouy *et al*., 1999), germination and seedling growth (Eisvand *et al.* 2011), precocity of flowering and yield (Kaur *et al*., 2005). Beneficial effects of seed priming has been shown in many crops including barley (El-Tayeb, 2005), maize (Farooq *et al*., 2008), sugar beet (Sadeghian and Yavari, 2004), sunflower (Demir Kaya *et al*., 2006), wheat (Iqbal and Ashraf, 2006),

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cotton (Casenave and Toselli, 2007) and chickpea (Eisvand *et al*., 2011; Azarnia and Eisvand, 2013). The use of biological fertilizers, especially plan growth promoting bacteria, is the most important strategy to increase production in sustained agricultural systems (Sharma, 2003). Arbuscular mycorrhizal fungi (AMF) (*Phylum glomeromycota*) are present in almost all the terrestrial ecosystems (Stürmer *et al*., 2013). They associate with the roots of more than 80% of the vascular plants, giving place to a mutual symbiosis denominated arbuscular mycorrhiza (AM) (Varma, 2008). The AM enhances the absorption of water and nutrients, mainly P (Stürmer *et al*., 2013). It also increases the tolerance of plants to biotic and abiotic stresses, as pathogens, drought and high salinity (Oztekin *et al*., 2013). Delian *et al*. (2011) claimed that the presence of mycorrhizas in soil can increase economic profitability and it is widely recorded that mycorrhizas influence crop productivity (Smith and Read 2008). Shaukat *et al*. (2006) stated that the races of Azosprilium, Pseudomonas and Azotobacter had significant effects on germination and seedling growth. Various experiments showed that inoculating plants with Azospirillium caused significant changes in different growth parameters such as increase in dry matter weight, nutrition absorption, tissue nitrogen, plant high, leaf size and root length in crops (Bashan *et al*., 2004). Various findings showed that seed priming improved emergence of seeds, root and seedling growth and finally suitable seedling establishment, so it seems that priming can provide conditions for better symbiotic relations between plant and micro-organisms. Literature review about interaction effect between priming and bio-fertilizers on crops such as lentil is low in this field, Alimadadi *et al*., (2010) reported that the combined effect of priming and mycorrhiza caused better nodulation, more positive effects on the percentage of active nodules and higher dry weight of nodules. Also most of the researches about lentil related to planting, but researches about interaction effect among mycorrhiza inoculation, seed priming and plant growth regulators especially in Iran is very low. Therefore, the purpose of this study was to evaluate effects of mycorrhiza inoculation and seed priming with plant growth regulators (GA3 and SA) on yield and yield components of lentil.

MATERIALS AND METHODS

A field experiment was conducted to study effect of mycorrhiza inoculation and seed priming with plant growth regulators on yield and yield components of lentil as factorial experiment based on the randomized complete block design with four replications in the research farm of Gonbad-e-Kavous University (Iran) during 2013-2014 growing season. First factor included inoculation of the mycorrhiza at three levels (control, inoculated soil using Glomus intraradices and Glomus mosseae) and seed priming at five levels (hydro-priming with distilled water, seed priming with GA_3 100 ppm, priming with SA 100 ppm, priming with GA_3 100 ppm + SA 100 ppm and non-primed seed as control) were second factor. Both mycorrhizal fungi (G. intraradices and G. mosseae) were collected from Zist Fannavaran Company, Iran. Lentil seeds of Kimiya which is well known by farmers of Gonbad Kavous were prepared from Agricultural Research Centre of Gonbad Kavous. For this experiment, seeds of lentil were disinfected using sodium hypochlorite (0.1%) then washed with distilled water for several times.

Priming

The disinfected seeds of lentil were maintained in priming solutions (GA3 100 ppm, SA 100 ppm, GA3 100 ppm+ SA 100 ppm) for 8 hours. An air pump was used for aeration during the priming process and then primed seeds were dried at 24°C. In case of mycorrhiza, 2×103 spore per gram was applied at the time of sowing in the soil. Distance of inter and intra row space of lentil seeds were 4 and 25 cm respectively. In this study, the distance of two replications were 1m. The research field was visited after seedling emergence and establishment daily.

Statistical analysis

Testing for data normality was done by SPSS software (version 20) firstly. Then variance analysis was performed using SAS software (Version 9.3) and the mean comparison which had significant differences have been compared with help of LSD test in 5% confidence level.

RESULTS AND DISCUSSION

Days to 50% flowering

Results showed that the main effect of priming and

Table 1: Data analysis of seed priming with mycorrhiza inoculation on lentil yield and its components Mean square.

S.O.V	df	days to 50% flowering	days to 50% podding	Number of pods per plant	Ratio of fill pod	Number of grain in pod	100 grain weigh	Grain yield	Biomass yield	Harvest index
Replication	3	45.75^{ns}	88.91^{ns}	932854.02^{ns}	1.19^{ns}	0.013^{ns}	7.43^{ns}	210513.66^{ns}	8285432.2**	67.79*
Mycorrhiza (A)	2	239.267**	439.43**	26301621.43**	14.43**	0.031^{ns}	25.45*	1748766.7**	8308285.3**	141.94**
Priming(B)	4	296.71**	477.08**	22059549.16**	24.16**	0.065*	32.14**	924008.36**	3400992.85**	75.44*
A*B	8	62.308*	56.07^{ns}	1375284.54*	9.83**	0.065**	57.83**	343565.1**	3764932.01**	80.32**
Error	42	25.51	33.55	600482.78	1.2	0.019	7.67	83075.68	680322.03	23.83
C.V (%)	-	5.15	5.47	13.6	13.81	11.42	5.80	12.94	11.70	15.23

**,*: Significant in 1 and 5% confidence level intervals respectively ns: non-significant difference.

mycorrhizal inoculant had a significant effect on days to 50% flowering of lentil in 1% confidence level. It was also observed that there was a significant interaction between mycorrhizal inoculation and priming (Table 1). On the basis of results, days to 50% flowering was significantly decreased with application of mycorrhizal inoculation along whole treatments of priming except G. mosseae in combination with both hormones level. The highest positive effect on this trait was found using treatment of G. intraradices+ GA100 ppm (85.25 day), but had no significant difference with application of G. intraradices+ hydropriming. Vosa´tka and Albrechtova (2008) reported that the use of mycorrhiza improved seedling survival, growth, seed yield and accelerated plant flowering. Rouphael *et al*. (2015) expressed that AMF improve nutritional status of plants by absorption and translocation of mineral nutrients beyond rhizospheric zone. In return, individual application of hormonal priming and hydro priming except GA100 ppm had no significant effect on days to 50% flowering (Table 2). Wu *et al*. (2014) reported that priming with salicylic acid decreased negative effects of salinity stress and the resistance of corn seedlings by increasing the content of carotenoids and antioxidant content of proline and soluble proteins and other osmolytes increased significantly. Kaur *et al*. (2005) reported that GA3 affects activity of different enzymes, especially amylase and increase mobilization of starch granules in cotyledons thus, stimulating germination and growth. Some researchers expressed that Seeds treat with GA_3 usually germination and grow faster have more developed root system, increase their tolerance to abiotic stress conditions, bloom and mature earlier and give better yields (Ekizce and Adak, 2005). Some reasearchers also reported that gibberellic acid hormone priming increased chick pea day to flowering; but abscisic asid decreased days to flowering (Azarnia and Eisvand, 2013).

Days to 50% podding

According to results, main effects of mycorrhiza inoculations and priming on trait of days to 50% podding were significant, while there was no interaction effect between mycorrhiza inoculation and priming aspect effect on the days to 50% podding (Table 1). Inoculation of seeds on days to 50% podding of lentil showed that mycorrhizal inoculant decreased this trait over control. The highest decrease effect was observed with application of *G. mosseae* (Fig 1). It was also observed various treatments of priming decreased days to 50% podding as comparison with control (Fig 2). Early flowering and days to 50% podding, enables plants to complete grain filling before drought stress at end of the season under rainfed conditions. These findings resemble with results of Djebali *et al.* (2010) which they reported that inoculation of chickpea, peas and alfalfa seed with mycorrhiza led to earlier flowering and podding. Azarnia and Eisvand (2013) indicated that seed priming stimulated early flowering and podding in chickpea.

Pods number per plant

Pods number is the most variable feature among pulses yield components. The effect of seed priming and mycorrhiza inoculation on the number of pods per plant as well as interaction between them were significant (Table 1). There were remarkable stimulation in pods number with application of *Glomus intraradices* in combination with hydro priming, although there was no significant difference between these treatments with some other treatments statistically. In return, the lowest number of pods was found with individual application of GA100 ppm about 2835 (Table 2).

Table 2: Mean comparison of mycorrhiza inoculation and priming on yield and components yield of lentil.

Mycorrhiza inoculation	Priming treatments	Days to 50% flowering	Number of pods	Ratio of filled pod	Number of grain in pod	100 grains weight (g)	Grain yield (Kg/h)	Biomass yield (Kg/h)	Harvest Index (%)
Non-Mycorrhiza	Control	106^{a}	4303^{bc}	8.79^{bc}	1.23^{bc}	46.45^{c}	2051^{bcd}	6700^{bcde}	31.03^{bc}
	GA$_{100}$ppm	94^{bc}	2835^{c}	9.84^{ab}	1.12^{c}	53.55^{a}	1780^{d}	5878^{e}	30.58^{bc}
	SA$_{100}$ppm	104^{a}	4794^{bc}	6.02^{ef}	1.18^{bc}	47.33^{bc}	1849^{d}	6406^{cde}	29.44^{c}
	Hydropriming	103^{a}	6372^{ab}	9.53^{ab}	1.58^{a}	43.83^{c}	1923^{cd}	6233de	30.64^{bc}
	SA + GA	102.3^{a}	4313^{bc}	5.56^{ef}	1.07^{c}	53.38^{a}	1892^{cd}	6494^{bcde}	29.67^{bc}
Glomus intraradices	Control	105.3^{a}	5937^{bc}	9.92^{ab}	1.15^{bc}	45.75^{c}	1910^{cd}	6622^{bcde}	29.45^{c}
	GA$_{100}$ppm	85.25^{d}	5643^{bc}	7.68^{cd}	1.2^{bc}	45.78^{c}	2222^{bc}	6850^{bcde}	32.45^{bc}
	SA$_{100}$ppm	102^{a}	7341^{ab}	6.06^{ef}	1.15^{bc}	46.15^{c}	2817^{a}	9329^{a}	30.22^{bc}
	Hydropriming	88.75^{cd}	9576^{a}	10.05^{ab}	1.12^{c}	54.08^{a}	3184^{a}	6800^{bcde}	47.61^{a}
	SA + GA	94^{bc}	5567^{bc}	10.24^{a}	1.17^{bc}	46.42^{c}	2227^{bc}	6378^{cde}	35.85^{b}
Glomus mosseae	Control	102^{a}	6248^{abc}	10.30^{a}	1.32^{b}	45.80^{c}	1919^{cd}	6961^{bcd}	27.82^{c}
	GA$_{100}$ppm	94.25^{bc}	4695^{bc}	6.41^{def}	1.1^{c}	50.63^{ab}	2334^{b}	7400^{bc}	31.65^{bc}
	SA$_{100}$ppm	95^{c}	6065^{bc}	5.43^{f}	1.22^{bc}	45.45^{c}	2416^{b}	7461^{b}	32.62^{bc}
	Hydropriming	95.5^{b}	7511^{ab}	6.40^{def}	1.2^{bc}	45.67^{c}	2838^{a}	9472^{a}	30b^{c}
	SA + GA	100^{ab}	4253^{bc}	6.89^{de}	1.1^{c}	45.72^{c}	2059^{bcd}	6733^{bcde}	31.78^{bc}
	LSD	6.40	3497	1.4	0.175	3.53	367.9	1053	6.23

Treatment means within columns followed by the same letter are not significantly different based on Least significant difference Test.

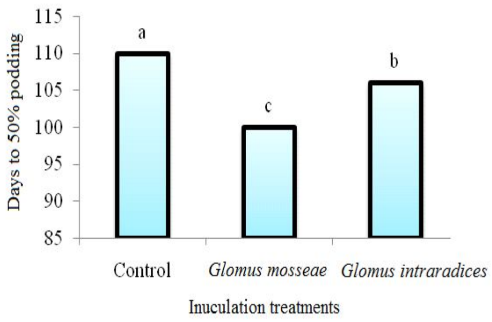

Fig 1: Effects of mycorrhiza treatments on podding of lentil.

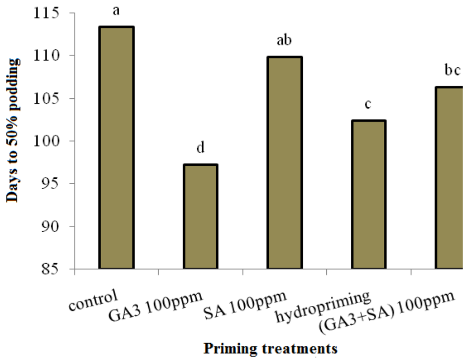

Fig 2: The effect of priming treatments on time to podding of lentil.

Ratio of filled pods to empty pods

Number of the pods, is the most important variable in the formation of grain yield of lentil. From the results, main effects of mycorrhiza inoculation and priming treatments and interaction of them were significant differences on ratio of filled pods to empty pods (Table 1). The highest ratio of filled pods to empty pods was found under individual application of *Glomus mosseae, G. intraradices* and hydro priming as well as combined treatment of *G. intraradices* with SA 100 ppm and GA 100 ppm statistically. While the lowest ratio of filled pod was found in the treatment of *G. mosseae* in combination with 100ppm salicylic acid (Table 2).

Number of grain per pod

According results, various treatments of priming had a significant impact on grain number in pods in 5% confidence level. But mycorrhiza inoculation had no significant effect on the studied trait. Overall, there was significant interaction effect between mycorrhiza inoculation and priming (Table 1). In the present study, the number of grain per pod was increased by hydro priming treatment about 28% over control only (Table 2). It has been reported that priming increased grain number per pod and per plant of pea (Azarnia and Eisvand 2013; Kaur *et al*. 2005). It was also observed that combined treatments of Glomus mosseae with GA 100 ppm and both SA and GA significantly decreased this trait as compared to control (Table 2).

100 grain weight

Generally, plants have mechanisms that regulate size of the sink based on the amount of available assimilate. From the results, effect of the mycorrhiza inoculation and priming and interaction effect between them on seed weight were significant (Table 1). Priming treatments of GA100ppm and SA 100 ppm + GA 100 ppm as well as combined treatment of *G. intraradices* with hydro priming improved the grain weight of lentil over control (Table 2). 100 grain weight is strongly affected by genetic and environmental factors and its amount is affected by the conditions of maturity period, these conditions may lead to 20 to 30 percent changes in 100-seed weight. Meera and Poonam (2010) stated plant growth regulator treatments significantly increased all physiological and yield components.

Biomass yield

According results, there was significant impact between mycorrhiza inoculation and seed priming on biomass yield of lentil in 1% confidence level (Table 1). This trait was significantly increased with application of Glomus intraradices with 100ppm salicylic acid and G. mosseae with hydro priming. The lowest biological yield was found in the treatment of GA 100 ppm. Although there was no significant difference between these treatments with some other treatments statistically (Table 2). Azarnia and Eisvand (2013) by investigating the effect of hydro-hormonal priming on yield and yield components of chick pea reported that hydro-priming increased chick pea biological yield, while priming whit gibberellic acid reduced chickpea biological yield.

Grain yield

Result showed that priming treatments, mycorrhiza inoculation and interaction between them were significant on grain yield in 1% confidence level (Table 1). Mycorrhiza inoculation with priming treatments improved grain yield significantly. The greatest stimulation effect was observed in the combined treatment of both mycorrhiza inoculation with and *G. intraradicis*+ hyro priming and *G. intraradicis* + SA 100ppm. Whereas individual application of priming had no significant effect on grain yield (Table 2). These results were in agreement with finding of Mohammadi *et al.* (2011). Some researchers reported that priming increased yield and yield components of chickpea (Azarnia and Eisvand, 2013), lentil (Das and jana, 2016; Ghassemi-Golezani *et al*., 2013) and soybean (Arif *et al*., 2014). Available literatures suggest that growth regulators can be used as potential tools to enhance the yield of pulses by minimizing the several physiological constraints (Singh *et al*., 2017). Ma *et al*. (1994) reported that Plant growth regulators (PGRs) have potential to increase chickpea yield and it may also increase protein levels of legume crops. Wheat seed priming improved plant stand and led to significantly higher grain yield (17%) over non-primed, in addition to significant difference between cultivars (Ramamurthy *et al*., 2015). This study also showed that maximum biomass yield and grain yield of lentil were obtained using mycorrhiza inoculation in combination with priming. Mehrbakhsh (1997) reported that lentil lines with high grain yield, have high biomass yield performance which this is similar to the results of this study.

Harvest index

Harvest index reflects the strength of genotypes to provide assimilate allocation to the seed destination (Mehrbakhsh, 1997). Study of Kumar and Srivastava (2015) showed that for creating change in lentil yield, it is critical to increment biological yield/plant, which had positive relationship with grain yield. Harvest index was significantly affected with application of mycorrhiza inoculation and priming (Table 1). The Harvest index was increased by most of the studied combined treatments. The best results were obtained when seeds were treated with *Glomus intraradices*+ hydro priming about 47.61% over control (Table 2). The development of early maturing genotypes with high harvest index is one of the important objective in lentil breeding program as early genotypes escape terminal drought (Kumar and Srivastava 2015). These results are agreement with results of Azarnia and Eisvand (2013). Overall, this study showed harvest index had a significant correlation with application of mycorrhiza inoculation and priming. Hashemi (2008) reported characteristics those were associated with grain yield components, improved harvest index and the second group of features didn't associate with seed yield and these features decreased harvest index.

CONCLUSION

Present study showed that in most of the treatments of mycorrhiza inoculation in combination with priming reduced the days to 50% flowering. It was also observed that combined treatments of Glomus intraradicis inoculation with hydro priming caused most early flowering, number of pods in plant, ratio of filled pod, grain weight of lentil eventually higher yields and harvest index of lentil over other treatments. Overall, the present findings indicate possibility of use of Mycorrhiza inoculation in combination with priming especially hydropriming to enhance productivity of lentil.

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