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Effects of seed deterioration and inoculation with *Mesorhizobium ciceri* on yield and plant performance of chickpea

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Abstract

Deterioration of seeds during storage can cause significant declines in seedling vigor and crop yield, particularly in less developed countries. This project compared seedling vigor and field performance of two chickpea (*Cicer arietinum*) cultivars (Arman and Hashem), subjected to deteriorating conditions, with or without subsequent rhizobial inoculation. Randomized seed groups were treated with 0 (control), 7, 14, 21 and 28 days of deterioration (DOD) in 40°C humid storage. Percent seed germination, radical length, seedling length, root weight and seedling weight were recorded. Seed germination and growth declined with increasing storage, and germination was zero after 21 or 28, DOD. Seeds with 0, 7, and 14 DOD were planted in field plots with and without inoculation with *Mesorhizobium ciceri*. At harvest plant height, biomass, filled and unfilled pod number per plant, seeds per plant, and seed yield were recorded. Increasing DOD led to decreasing growth and yield. Hashem variety yields were reduced 17% and 37% by 7 and 14 DOD, respectively, less than Arman which was reduced 40% and 42%, respectively. This suggests that Hashem cultivar can be stored in conditions of high heat and humidity with less reduction in yield. Inoculation of seeds with *M. ciceri* prior to planting improved yield and plant biomass in both varieties by 6-36%. However the benefit of inoculation was only significant in Hashem at 0 and 7 DOD and in Arman at 0 DOD. These results show that rhizobial inoculation has the greatest benefit on healthy seeds but can also diminish the yield decrease caused by non-ideal storage conditions.

Keywords: Biofertilizer, Chickpea, Seed Deterioration, Germination, *Mesorhizobium ciceri*
Abbreviation: DOD= days of deterioration

Introduction

Chickpea (*Cicer arietinum*) is one of the most important pulse crops, providing high quality protein for human nutrition around the globe. In 2008, 92% of the world's chickpeas were produced in developing countries (FAO, 2009; IMF, 2010). During seed storage between field seasons, seed stocks can suffer deterioration due to heat, humidity, and biotic agents. Storage of seed in developing areas like Iran is often less than ideal, and deterioration of seed can be significant. Sustainable mechanisms to improve seed storage and reduce the impact of seed deterioration on yield are needed to improve global food security. Seed deterioration is a loss of viability, vigor, and overall seed quality due to aging or adverse environmental factors. High seed moisture content and storage temperature are among the most critical factors in seed deterioration (Ellis et al., 1985). The use of deteriorated seeds may lead to a yield decrease for two reasons. The percentage emergence of deteriorated seeds is less than that of healthy seeds. Therefore, deteriorated seed often produces uneven stands, spotty fields, and fewer plants per hectare than healthy seed. The subsequent plant growth rate can also be reduced in plants that have originated from deteriorated seed. Kapoor et al., (2010) reported seed quality in chickpea deteriorated following accelerated ageing treatment of 45 °C and 100% humidity for 24, 48, or 72 hours. With increasing time of ageing treatment, seed moisture content increased and all physiological parameters measured such as germination percentage, seedling root length, shoot length, and vigor index decreased in all varieties. Ageing treatment also reduced seed protein and sugar content. There were significant differences in the physiological and biochemical deterioration responses among the 5 chickpea varieties used. *Mesorhizobium ciceri* inhabit nodules of chickpea roots and supply plant-available nitrogen through nitrogen fixation. The inoculation of chickpea seed with symbiotic rhizobial strains improves nodulation, yield, and biomass (Rennie et al., 1986). Rhizobial inoculation effects have not been studied on deteriorated seed. In this study, chickpea seeds were subjected to deterioration treatment and then grown with or without rhizobial inoculation.

Materials and methods

Seed Deterioration

The effect of seed deterioration on seedling germination was investigated in a lab experiment. Seeds of Hashem and Arman cultivar chickpeas were subjected to 0, 7, 14, 21 or 28 days of deterioration (DOD). The deterioration treatment was as follows. One kg of seeds was placed in mesh bags. Each bag was placed on a sieve suspended above water within a closed chamber held at 40ºC. The bags did not contact the water at any point, but seeds could absorb the humidity in the chamber. After the proper number of days of treatment the seeds were placed between 2 sheets of moist germination paper for testing germination. We then rolled the paper, tied it with a rubber band, placed each in a plastic bag, and placed these in a germination box at 20º C and 70% humidity for 10 days. We determined the seed germination percentage, radical and seedling length, radical and seedling weight.

Field Performance

The field work was conducted at the research farm of Gonbadekavoos High Education Center, Iran, latitude 37˚ and 16' and longitude 55˚ and 12'. Average temperature is 17.7˚ C and average annual precipitation is 487 mm. The soil was a loamy clay with pH 7.7. The experiment was a factorial completely randomized design with 3 factors and 3 replicates. Factors included cultivar (Hashem and Arman), inoculation (with or without *Mesorhizobium ciceri*) and deterioration (7 and 14 days and control). After 21 and 28 DOD seeds did not germinate in the lab, so only seeds subjected to 0, 7 and 14 DOD were used for the field experiment. All field activities including planting, irrigation, pest protection, weed control, and harvest were similar to the practices used by farmers in the area of Gonbadekavoos. Seeds in the inoculated treatment were mixed with *M. ciceri* inoculum 1 hour prior to planting at the rate of 1 L ha-1. Conservation tillage was used to prepare the seed bed, and herbicides (Sonalan®, active ingredient ethalfluralin, and Treflan®, active ingredient trifluralin) were used to control weeds. The area of each plot was 2.5 m^2, 5 rows in each plot, and 50 cm between rows. Seeds were planted at approximately 1-2 cm intervals and plants were thinned to 7 cm between plants at the 4-5 leaf stage. We measured plant height, filled and unfilled pod number per plant, seed number per plant, total plant dry weight and seed yield at harvest.

Statistical Analyses

Data were analyzed as a factorial completely randomized design with 3 factors and 3 replicates using ANOVA in SAS (SAS Institute, 1990). Effects were considered significant at P values ≤ 0.05. Duncan multiple range tests were conducted for mean comparison.

Results

Seed Deterioration

Cultivar, deterioration treatment, and interaction between cultivar and deterioration significantly affected seed germination, cotyledon length, cotyledon weight, radicle length, and radicle weight (Table 1). The control (non-deteriorated) seeds performed best in all germination and seedling characteristics (Table 2). Seeds of both varieties declined in germination and growth with increasing deterioration. After 21 or 28 DOD no seeds germinated (data not shown). Seven and 14 DOD reduced germination on average by 57% and 80%, respectively. After 14 DOD only radicles were present on 10-day old germinated seedlings. Seedlings of the cultivar Hashem generally performed better than Arman (Table 2). In non-deteriorated seed Hashem produced more cotyledon length and radicle length and weight than Arman. After 7 DOD germinated Hashem seed produced more cotyledon length and weight than Arman, but less radicle weight. Response to 14 DOD was similar in both cultivars.

Field Performance

Because seeds of both varieties failed to germinate after deterioration greater than 14 days, only non-deteriorated, 7, and 14 DOD seeds were followed in a field study. Plant height, filled pod number, unfilled pod number, seeds per plant, seed weight, yield, and plant dry weight were generally significantly

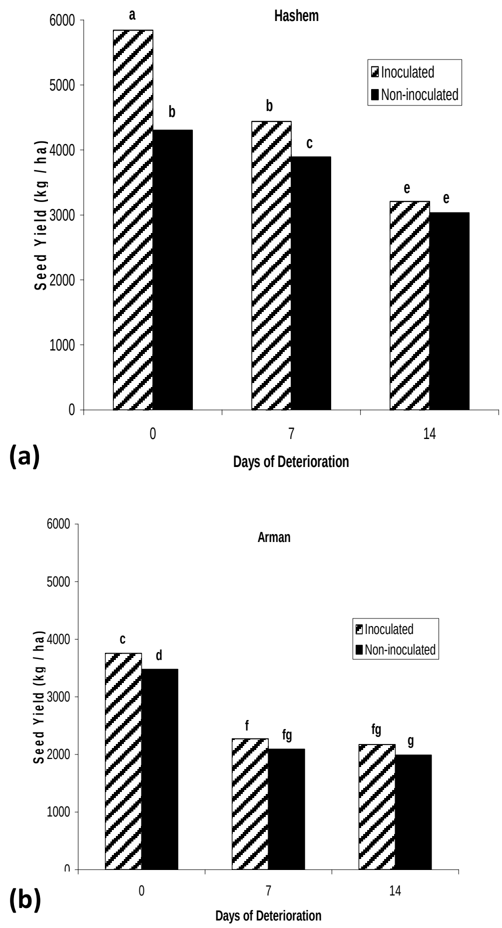

Fig 1. Seed yield of a) Hashem and b) Arman variety chickpeas treated with 0, 7, or 14 days deterioration stress, with or without inoculation with *Mesorhizobium ciceri*. Bars with different letters are significantly different at P < 0.05.

Table 1. Analysis of variance of germination percent, cotyledon length and weight, radicle length and weight of Hashem and Arman variety chickpeas after 0, 7, or 14 days storage at 40°C.

Sources of Variation	Degrees of freedom	Mean squares				
		Germination percent	Cotyledon length	Cotyledon weight	Radicle length	Radicle weight
Cultivar (C)	1	133**	6.1**	0.0007*	3.5**	0.0001**
Deterioration (D)	2	1221**	7.1**	0.025**	25**	0.02**
C × D	2	49**	1.6**	0.0001*	1.9**	0.004**
Error	12	10.6	0.03	0.00008	0.19	0.00007
Coefficient of Variation		6.6	17.6	11.3	12.2	12.7

Table 2. Means of germination percent, cotyledon length and weight, radicle length and weight of Hashem and Arman variety chickpeas after 0, 7, or 14 days storage at 40°C.

Cultivar	Deterioration (days)	Germination (%)	Cotyledon length (cm)	Cotyledon weight (g)	Radicle length (cm)	Radicle weight (g)
Hashem	0	100 a	3.1 a	0.14 a	6.2 a	0.16 a
	7	42 b	1.8 b	0.10 b	2.7 c	0.02 d
	14	19 c	0.0 d	0.0 d	1.1 d	0.03 d
Arman	0	100 a	1.3 c	0.12 a	4.0 b	0.10 b
	7	43 b	0.14 d	0.08 c	2.2 c	0.07 c
	14	21 c	0.0 d	0.0 d	1.1 d	0.02 d

Values within the same column followed by the same letters are not significantly different according to Duncan's multiple range test (*P* =0 .05).

Table 3. Analysis of variance of plant height, filled pod number, unfilled pod number, seeds per plant, seed weight, yield, and plant dry weight, of Hashem and Arman variety chickpeas after 0, 7, or 14 days storage at 40°C, with and without inoculation of *M. ciceri*.

Sources of variation	Mean squares					
	Plant Height	Filled pods	Unfilled pods	Seeds per plant	Yield	Dry Biomass
Cultivar (C)	132**	163**	0.6	351**	200653**	725**
Inoculated (I)	0.3	7**	18**	44**	21003**	265**
Deterioration (D)	697**	609**	329**	756**	94724**	147**
C × I	0.04	0.9	1.3	24**	6547**	126**
C × D	96**	38**	2.9**	26**	6723**	8.7**
I × D	10.5**	1.3*	7.5**	7.8**	4274**	12**
C × I × D	4.0**	1.7*	2.1*	8.7**	3200**	17**
Error	0.58	0.38	0.4	0.6	173	0.18
Coefficient of variation	1.2	3	4.7	3.1	3.9	2.8

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 4. Means of chickpea growth and yield components as affected by main effects of cultivar, inoculation, and deterioration treatment.

Treatment		Plant height (cm)	Filled pods	Unfilled pods	Seeds per plant	Yield (kg ha^{-1})	Dry Biomass (g m^{-2})
Cultivar	Hashem	61.6 b	22.7 a	13.4 a	28.3 a	4197 a	19.2 a
	Arman	65.4 a	18.5 b	13.6 a	22.1 a	2626 b	10.9 b
Inoculation	Inoculated	63.6 a	21.1a	12.8 b	26.3 a	3614 a	18.1 a
	Non-inoculated	63.4 a	20.2 b	14.2 a	24.1 b	3131 b	12.7 b
Deterioration (days)	0	70.7 a	28.6 a	17.8 c	33.1 a	4344 a	19.2 a
	7	64.3 b	18.3 b	15.0 b	25.1 b	3174 b	14.9 b
	14	55.5 c	14.9 c	7.7a	17.2 c	2601 c	12.2 c

Values within the same column followed by the same letters are not significantly different according to Duncan's multiple range test (*P* =0.05)

Table 5. Means of chickpea growth and yield components as affected by the interaction of cultivar, inoculation, and deterioration treatment.

Cultivar	Inoculation	Deterioration (days)	Plant height (cm)	Filled pods	Unfilled pods	Seeds per plant	Yield (kg ha^{-1})	Dry Biomass (g m^{-2})
Hashem	Inoculated	0	66.8 b	34.1 a	6.8 g	41.7 a	5841 a	31.2 a
		7	60.0 d	19.7 d	14.0 e	29.0 c	4439 b	24.0 b
		14	58.2 e	16.2 ef	16.5 c	19.9 f	3208 e	18.3 c
	Non-inoculated	0	66.3 b	31.4 b	7.4 g	34.1 b	4304 b	17.6 c
		7	62.8 c	19.7 d	16.8 c	26.4 d	3893 c	15.2 d
		14	55.4 f	15.4 fg	18.6 b	18.6 g	3033 e	13.3 f
Arman	Inoculated	0	74.4 a	24.6 c	8.8 f	28.5 c	3753 c	14.4 e
		7	67.1 b	17.2 e	14.3 e	23.1 e	2273 f	11.4 g
		14	55.1 f	14.5 gh	16.4 c	15.4 h	2173 fg	9.6 h
	Non-inoculated	0	75.4 a	24.3 c	7.56g	28.2 c	3479 d	13.4 f
		7	67.1 b	16.7 e	15.2 d	22.0 e	2091 fg	9.1 h
		14	53.4 g	13.6 h	19.7 a	15.1 h	1990 g	7.7 i

Values within the same column followed by the same letters are not significantly different according to Duncan's multiple range test (*P* =0.05).

affected by the main effects of cultivar, deterioration, and inoculation, as well as by interactions of the main effects (Table 3). Overall, Arman produced taller plants but Hashem produced 60% greater yield and 75% greater total biomass (Table 4). Hashem outproduced Arman whether non-deteriorated or deteriorated seeds were used. On average, 7 and 14 DOD reduced chickpea yield by 27% and 40%, respectively, as compared to non-deteriorated seed (Table 4). However this decline was not equal between varieties. Hashem variety yields were reduced 17% and 37% by 7 and 14 DOD, respectively, less than Arman which was reduced 40% and 42%, respectively (Table 5, Figure 1). Non-deteriorated and inoculated seeds generally produced the greatest plant height, filled pods, seed yield, and plant biomass (Table 5). The greatest seed yield was obtained from Hashem cultivar, inoculated and without deterioration (5841 kg ha^{-1}), and lowest seed yield was obtained from Arman cultivar, without inoculation after 14 DOD (1990 kg ha^{-1}) (Table 5). Filled pod number, seeds per plant, and plant biomass were also all greatest in non-deteriorated inoculated Hashem cultivar and least in non-inoculated Arman with 14 DOD (Table 5). The difference in crop performance between maximum and minimum seed yield was 66%, showing the importance of cultivar, deterioration and inoculation in the cultivation of chickpeas. Inoculation of seeds with *M. ciceri* prior to planting in the field improved all plant characteristics except plant height (Table 4). Inoculated plots produced on average 15% greater yield across all deterioration treatments and both cultivars (Table 4). Inoculation increased plant biomass in both cultivars using non-deteriorated seeds and after 7 or 14 DOD (Table 5). Chickpea yield, however, was only significantly increased in both varieties of non-deteriorated seed and in Hashem after 7 DOD (Figure 1). The greatest yield benefit of inoculation (36%) was seen in the non-deteriorated seed of Hashem. Yield of Hashem with 7 and 14 DOD increased 14% and 6% with inoculation, respectively. The yield of inoculated Hashem with 7 DOD was greater than the yield of non- inoculated Hashem even without deterioration. All treatments within Arman variety had only 8-9% greater yield with inoculation.

Discussion

Deterioration by high heat and humidity causes damage to cell membranes, destroys the activities of enzymes, and prevents cellular building (Hampton and Tekrony, 1995). Other biochemical changes during chickpea seed deterioration or ageing processes include declines in protein and sugar (Kapoor et al., 2010). We found that increasing length of deterioration treatment reduced all measures from seed germination and growth in lab trials to crop production in field trials (Tables 2, 4), in agreement with Rouz rokh et al., (2002). These results show that with increasing deterioration the seed health decreases, as well as seedling and crop performance of surviving plants. Hashem variety was much more resilient to deterioration treatment than Arman variety. Seed germination and most measures of seedling growth were similar between varieties, but cotyledon length in Hashem after 7 DOD was significantly greater than in Arman. In the field, the relative yield decline in Hashem variety after 7 DOD was less than half as severe as the yield decline in Arman variety after 7 DOD. This suggests that Hashem cultivar can be stored in conditions of high heat and humidity with less reduction in yield, if seeding rates are increased to account for lower germination. Kapoor et al., (2010) also found that seed deterioration under hot and humid conditions varied significantly among chickpea cultivars. The positive yield and biomass benefits of *M. ciceri* on chickpea are well established given good field conditions (Rennie and Dubetz, 1986; Yahiya et al., 1995). In the cultivar-by-deterioration treatments of this study, inoculation improved yield of deteriorated seeds by 6-14% whereas 7 DOD reduced yield 10-40%. These results show that proper rhizobial inoculation can help to mediate the negative effects caused by non-ideal seed storage conditions, but the benefit of *M. ciceri* could be expected to remediate the destructive effects of only a few days of extreme temperature and humidity. A few other studies have also found benefits of rhizobial inoculation given non-ideal conditions such as fungal pathogen pressure (Sindhu et al., 2002) or salt stress (Mhadhbi et al., 2004). However there is precedent for the finding that rhizobial inoculation is most beneficial in ideal conditions. Gan et al., (2008) found that inoculation provided the most benefit to chickpeas grown at soil moisture of 90% field capacity, increasing seed yield by 121%.

At 60% and 30% field capacity, inoculation increased seed yield only 4-5%. Similarly, adequate phosphorus nutrition is necessary to support maximum N fixation in chickpea (Yahiya et al., 1995). While nitrogen fixation clearly can improve chickpea yield, this service does come at a cost of nodule formation and photosynthate supplied to nodules. The plant must be healthy and otherwise able to support its N fixing symbiont in order to gain maximum benefit from the interaction.

Conclusions

Sustaining and increasing production of high quality protein foods for the growing human population will require efficient use of resources in both ideal and non-ideal conditions. High seed quality should be maintained to minimize the quantity of seed and other resources needed for planting and production. However, given that many regions do not have ideal seed storage facilities, information is needed about crop varieties that maintain seed health in deteriorating conditions, as well as potential seed treatments to counteract deterioration. In this study we found that Hashem variety cultivar had not only good field performance by non-deteriorated seed, but substantial resilience to seed deteriorating conditions. Inoculation of chickpea seeds with *M. ciceri* provided the greatest benefit to non-deteriorated Hashem seed, but also provided moderate remediation of deterioration treatments. Increased use of varieties that withstand non-ideal seed storage conditions, and consistent use of proper rhizobial inoculants, should be recommended particularly for regions without ideal storage facilities.

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