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Effect of Seed Biopriming on Germination and Growth of Chickpea (*Cicer arietinum* L.) Under Water Stress

[1] Khadraji Ahmed, [2] Qaddoury Ahmed, [3] Ghoulam Cherki

[1, 3] Center of Agrobiotechnology and Bioengineering, (Centre AgroBiotech-URL-CNRST-05), Laboratory of Biotechnology and Agrophysiology of Symbiosis, Cadi Ayyad University, Marrakesh, Morocco

[2, 3] Agrobiosciences Program, University Mohamed VI Polytechnic, Benguerir, Morocco

Corresponding Author: **Khadraji Ahmed**

Abstract

Chickpea is a source of energy, protein, vitamins, minerals, and fiber and without cholesterol. Chickpea makes it possible to cover the protein deficit of the Indian and Mediterranean populations. The aim of our work is to study the effects of biopriming, which is a technique of priming seeds with living microbial cells (in our case we used rhizobium isolates) on germination and growth under water stress. The chickpea varieties used in the experiment are Farihane, Zahor, Rizki, and Bouchra. After 7 days, the biopriming seedlings were separated into two lots in a growth chamber with irrigation at 40% and 80% of field capacity. The results showed an increase in germination parameters such as the germination percentage, mean germination time and T50. Treatment of Zahor seeds with rhizobia improved germination percentage by 15% compared to unprimed seeds. For the varieties Farihane and Zahor, biopriming improved the MGT by 37 and 42% respectively compared to the unprimed seeds. For the variety Zahor, plant growth resulting from biopriming decreased by only 9% under severe water deficit. In conclusion, biopriming with Rhizobium isolates improved all studied germination and growth parameters.

Keywords: Biopriming, Chickpea, Germination, Rhizobia

Introduction

The legume family is vast, consisting of many species of peas, beans and chickpeas, and plays an important role in ecology, agriculture and economy (De Ron, 2015) [6]. Chickpea is a crop that will become increasingly important with climate change (Merga and Haji, 2019) [19]. Indeed, the nutritional value of chickpea in terms of nutrition and particularly its high protein content have made chickpea a key food in human nutrition in developing countries (Malunga *et al.*, 2014) [17]. Moreover, the chickpea, like all legumes, is a food naturally rich in vitamins and without cholesterol; it is widely used in vegetarian recipes (Mefleh *et al.*, 2022) [18]. The world production of chickpea crop ranks third after beans with an annual production of 11.5 million tons, most of the production is concentrated in India. The area under chickpea is 14.56 million hectares. More than 2.3 million tons of chickpeas are produced each year. In Morocco, the chickpea occupies the second position after the bean and consists mainly of the Kabuli type (Khadraji *et al.*, 2020) [10].

The interest of legumes lies in the particularity of forming symbioses with Rhizobia (Concha and Doerner, 2020) [5]. The nodule within which nitrogen fixation takes place, which is summarized by the reduction of atmospheric nitrogen N_2 in ammonia form (Lindström and Mousavi, 2020) [13]. In addition, their inclusion in cropping systems improves soil fertility and allows for a reduction in fertilizer use for subsequent crops. This reduction in the use of nitrogenous fertilizers results in a reduction in greenhouse gas emissions (Boddey *et al.*, 2020) [2].

Drought is among the most limiting abiotic factors in global production, water deficit starts at the root level by directly affecting the water absorption process (Knipfer and Fricke, 2010) [11]. This constraint causes the reduction of aerial growth and different physiological and biochemical changes (Mouradi *et al.*, 2016; Khadraji *et al.*, 2017) [9]. The water deficit can also affect phosphate nutrition in semi-arid areas by drastically reducing the possibilities of desorption of phosphate ions from the solid phase of the soil and their transfer to the root. Indeed 95% of the phosphorus taken must be transformed before being transferred to the plant (Rouphael *et al.*, 2012) [25].

Priming is one of the best-known pre-seeding techniques for influencing seedling development by modulating the metabolic activities of germination before radicle breakthrough. That is, during the reversible phase of germination, the seed can be rehydrated while retaining its ability to germinate (Lutts *et al.*, 2016) [14]. During priming, primed seeds become activated more rapidly and begin the metabolic processes associated with germination initiation (Mouradi *et al.*, 2016; Khadraji *et al.*, 2017) [9]. In addition, seed priming stimulates various metabolic processes that enhance seed germination and allows the germination of dormant seeds, particularly legume seeds (Pawar and Laware, 2018) [24]. There are different seed priming techniques like hydropriming, halopriming, osmo-priming, hormone-priming, thermopriming, matrix-priming and solid-priming (Mouradi *et al.*, 2023) [21]. Biopriming is a combination of seed priming and bacterial inoculation, it is a simple, economical and effective technique to improve the tolerance and establishment of plants in a stressful environment (Mahmood *et al.*, 2016; Lahrizi *et al.*, 2021) [15, 12]. Applied treatment of plant growth stimulants, rhizobacteria (PGPR) after seed priming may have the ability to counteract oxidative damage under water stress and improve stress tolerance for better crop yield (Pawar and Laware, 2018) [24]. PGPR possesses the enzyme 1-aminocyclopropane-1-carboxylateeaminase (ACC)-deaminase, which inhibits the plant retarding hormone-ethylene and promoted antioxidant defense system to improve tolerance against terminal water stress in legume, crops (Glick *et al.*, 2007) [8]. The objective of this work was to study the effect of biopriming on the germination and growth of some chickpea varieties.

Materials and Methods

The experiment was conducted in Laboratory of Biotechnology and Agrophysiology of Symbiosis. Seeds of four varieties of chickpea (Farihane, Zahor, Rizki and Bouchra) from INRA Settat were surface disinfected with 1% solution of sodium hypochlorite for 2-3 minutes and rinsed several times. Nodules recovered from chickpea roots were sterilized by ethanol, followed by rinsing in distilled water. Then, the bacterial isolates were spread in a petri dish containing YEM medium for 48 hours at 28°C. Colonies in each dish were suspended in 20mL of Carboxy Methyl Cellulose (CMC) and pectin solution used as adhesive polymers for the bio-priming process of chickpea seeds (Sufyan *et al.*, 2020) [27]. The seeds were dried in moist chamber for 24 hours. Chickpea seeds only treated with Carboxy Methyl Cellulose solution taken as control. After 7 days of germination in petri dishes, the seeds were transplanted into a culture room (28 °C). The seedlings were subjected to two treatments (40%) and a control at 80% of field capacity (FC). Water stress was applied to plants for a 40 day period; the length of the aerial part was measured (cm).

Germination percentage (GP %): it represents the final number of germinated seeds (n) in proportion to the total number of seeds sown (N) in each Petri dish and was calculated according to the following formula by Onofri *et al.* 2018 and expressed in %. $(GP) = (n / N) \times 100$

Mean germination time (MGT): It was calculated according to the equation of Ellis and Roberts (1981) [7] and expressed in days. The equation is as follows: $MGT = \sum D n / \sum n$

Where, "D" is the number of days counted from the beginning of the test and "n" is the number of seeds that germinate on day 'D'.

Time to 50% germination (T50): T50 was defined as days needed to reach 50 per cent of final germination percentage. T50 was calculated according to the formula of Coolbear *et al.* (1990) [4] using the following formula: $T50 = ti + [(N / 2) - ni) (ti-tj)] / (ni-nj)$

Where, N is the final number of germination and ni, nj cumulative number of seeds sprouted by adjacent counts times ti and tj when ni <N / 2 <nj.

Statistical Analysis: After 7 days, the parameters of germination were recorded. The data was statistically analyzed using SPSS (21.0) software. Two-way analysis of variance (ANOVA II) were performed using three replicates per combination per treatment for almost all studied parameters. The treatment means were compared by ANOVA and the least significance difference test was applied for checking the significance of values (P≥0.05).

Results and Discussion

Effect of Biopriming Chickpea Seeds on Germination Percentage

Germination percentage increased significantly (P< 0.001, Table 1) in the treated varieties compared to their respective controls. The highest PG values was noted in the rhizobium treated varieties Zahor and Farihane with a germination percentage (100%) compared to their respective controls (85% and 95%). On the other hand, the variety Rizki presented the lowest value (85%) in the unprimed seeds. Biopriming significantly improved the germination percentage of seeds of different species such as Canola seed (Ataei *et al.*, 2016) [1], *Brassica rapa* seeds (Chin *et al.*, 2021) [3] and chickpea seeds (Yadav *et al.*, 2013) [28].

Effect of Biopriming Chickpea Seeds on MGT

Mean germination time (MGT) was significantly different (P< 0.001, Table 1) in the treated varieties compared to their respective controls. The MGT of all seeds primed with bacterial isolates was improved (between 17 and 41%) compared to their controls. For the varieties Farihane and Zahor an improved MGT of 37 and 42% respectively was recorded. Biopriming of seeds stimulates the mobilization of reserves and consequently reduces the germination time and improves the germination percentage (Makhaye *et al.*, 2021) [16].

Effect of Biopriming Chickpea Seeds on T50

The time for 50% of seeds to germinate varied significantly (P< 0.001, Table 1) in the treated varieties compared to their respective controls. Most of the seeds of the varieties (Zahor, Rizki and Farihane) primed by bacterial isolates improved T50 (41%, 34 and 44%, respectively) compared to their controls. For the Bouchra variety, an increase in T50 of 3% was noted. Biopriming had a positive effect on T50 which is in agreement with the work of Sowmya *et al*,(2022) [26] who noted that T50 and mean germination time (MGT) were also lower (1.42 days and 1.77 days) in seeds primed with phosphorus solubilizing bacteria (PSB).

Table 1: Effect of seed biopriming (UP: Control and BioP: primed seeds) on germination percentage, mean germination time (MGT) and T50 in four chickpea varieties (Bouchra, Zahor, Rizki and Farihane). Values are means of three replicates ± standard errors

Varieties	treatment	PG%		MGT (d)		T50 (d)	
Bouchra	UP	95[bc]		4.78± 0.12[abc]		4.384± 0.057[ab]	
	BioP	90[ab]		4.65±0.050[bcd]		4.396± 0.019[ab]	
Zahor	UP	85[abc]		4.91± 0.027[a]		4.470± 0.038[a]	
	BioP	100[a]		4.53 ±0.041[a]		4.276± 0.021[d]	
Rizki	UP	85[c]		4.81± 0.105[ab]		4.491 ±0.079[a]	
	BioP	92.5[ab]		4.58± 0.159[cb]		4.323± 0.025[bc]	
Farihane	UP	95[abc]		4.87 ±0.098[a]		4.422± 0.069[b]	
	BioP	100[a]		4.55± 0.051[d]		4.237± 0.010[d]	
		df	f	df	f	df	f
Varieties		7	6.04***	7	8.5***	7	11.4***
Error		16		16		16	

Values followed by a different letter are significantly different at $p < 0.05$.

Length of Aerial Part

The results obtained showed that in the seedlings resulting from biopriming, the length of the aerial part of the Bouchra variety decreased only by 11% compared to the control (29%) under water deficit 40% of field capacity. For the variety Rizki, the length of the stem decreased, only by 5% in the plants resulting from biopriming compared to the control (12.5%) under severe water deficit. For the variety Zahor, the length of the aerial part of the plants resulting from biopriming decreased only by 9% under severe water deficit compared to the control (21%). According to Moualeu-Ngangué *et al.*(2020), water stress has a negative effect on plant morphology, including a reduction in dry mass, leaf area and stem length.

Conclusion

The biopriming effect was positive at the germination stage; the primed seeds were faster to germinate with a higher percentage of germination compared to untreated seeds. Biopriming also allowed the seedlings to develop well under water stress. In fact, the reduction in growth was greater in plants from untreated seeds under water stress. The biopriming technique has several advantages such as low cost, very practical and guarantees the growth of plants and their adaptation to water stress conditions.

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