RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 7: 793-797 (July 2021)

The Relationship between Seed Vigor and Germination Performance under Various Chloride Salts in Pea

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10.18805/LR-559

ABSTRACT

Background: Salinity is a severe abiotic stress resulting in inhibiting germination, seedling growth of crop plants. Germination performance under salinity depends on seed vigor and genotypic factors. This study aimed to determine if there was a relationship between germination and seedling growth of eight pea cultivars subjected to three chloride salts and the results of seed vigor tests.

Methods: Pea cultivars Ambassador, Bolero, Electra, Esprit, Puccet, Rainer, Utrillo and Winner were germinated in the medium with three chloride salts of NaCl, $MgCl_2$ and $CaCl_2$ with the electrical conductivity (EC) of 20 dS m^{-1}. Also germination percentage, mean germination time, controlled deterioration (CD) and electrical conductivity (EC) test were evaluated.

Result: The seed vigor of pea cultivars was significantly different and salinity led to a significant decline in parameters of the cultivars. The most hazardous salt was $MgCl_2$, while the least harmful was NaCl. Germination percentage was negatively related with MGT and EC values and EC test was strongly correlated with CD test. Utrillo and Winner were the most sensitive cultivars to $MgCl_2$ and $CaCl_2$. Although germination percentage of Rainer under non-saline was the lowest with 76%, it showed better performance under salinity than Utrillo and Winner. Germination percentage and CD gave a negative significant correlation with NaCl, but MGT and EC associated positively with NaCl. It was concluded that seed vigor should be considered as an indicator for the germination performance of pea seeds under NaCl, whereas genotypic factors play a key factor for tolerance to salinity during germination and early development stages.

Key words: Correlation, *Pisum sativum* L., Salinity, Seed vigor.

INTRODUCTION

Fresh beans and grains of peas (*Pisum sativum*) are commonly used as vegetable and canning industry in Turkey (Bozoglu *et al.* 2004). It is an important crop as cheap protein sources with 20-30% protein content in seeds (Öz and Karasu, 2010). It is a cool season food legume that allows early sowing in spring to produce high fresh bean and dry grains under arid and semi-arid conditions (Kelly, 1987). Because pea is particularly sensitive to drought (Wilson *et al.* 1985) and salinity stresses (Lal, 1985) during early growth stages, tolerant varieties provide considerable advantages under these conditions (Dudhe and Kumar, 2018). The pea cultivars had significant variation for salinity tolerance during germination and early seedling development (Cerda *et al.* 1982 and Okçu *et al.* 2005).

A single individual pea plant produces different seeds in their appearance and spatial heterogeneity of the pod position causes considerable variations in shape, size, weight, maturity and color of the seeds (Fenner, 1993; Coste *et al.* 2005). These differences may affect the physiology and viability performance of the seeds (Kahn *et al.* 1996). Atak *et al.* (2008) reported that the seed vigor of pea cultivars changed with seed color in the cultivars and they tested the seed vigor by electrical conductivity and accelerated ageing tests. The electrical conductivity (EC) test has been recommended for garden peas by ISTA (2003) as a vigor test. It is a simple, quick and effective method measuring indirectly the cellular membrane damage due to seed deterioration by determining electrolytes leakage from seeds immersed in distilled water. Taweekul *et al.* (1998) and Atak *et al.* (2008) determined a negative relationship between electrical conductivity values and seed vigor in pea. Also, controlled deterioration (CD) is another vigor test permitting the seeds to increase their moisture content up to 20-24% before the seeds expose to high temperatures between 40 and 45°C for 24 h or above. This test showed the superiority for distinguishing seed vigor in several plants such as onion by Rodo and Marcos Filho (2003), in cabbage by Mathews *et al.* (2009) and in wheat by Modarresi and Van Damme (2003).

The relative importance of seed vigor in salinity tolerance during germination in pea is not clearly understood and this study was arranged to find the relationship between seed vigor and germination and seedling development of pea under chloride salts.

MATERIALS AND METHODS

The certified seeds of pea cultivars Ambassador, Bolero, Esprit, Electra, Puccet, Rainer, Utrillo and Winner were used

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How to cite this article: Kaya, G. (2021). The Relationship between Seed Vigor and Germination Performance under Various Chloride Salts in Pea. Legume Research. 44(7): 793-797. DOI: 10.18805/LR-559.

Submitted: 20-03-2020 **Accepted:** 27-02-2021 **Online:** 31-03-2021

in the study. They were obtained from commercial seed suppliers and were produced in 2016 under Eskişehir conditions to have uniform seeds in terms of seed vigor. Three salt types, sodium chloride (NaCl), magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) with the electrical conductivity of 20 dS m^{-1} were arranged at temperature of 20°C by the conductivity meter WTW 3.15i (Germany) at Seed Science and Technology Laboratory, Field Crops Department, Eskişehir Osmangazi University. The control treatment was exposed to distilled water (~0 dS m^{-1}).

Germination test

Four replicates of 50 seeds (totally 200 seeds) were used for each cultivar and salt type. Fifty seeds were inserted into three layers of filter papers with dimension of 20 × 20 cm moistened with 21 mL of the solutions. The papers were renewed every 2 days to avoid the accumulation of salt (Kaya *et al.* 2008). After the papers were rolled, they were embedded a sealed polyethylene bag to prevent water evaporation. The seeds were incubated at 20±1°C in the darkness for 10 days. A 2 mm of root protrusion was evaluated as germination criterion. Germination speed was calculated for the mean germination time (MGT) as described by ISTA (2003) rules with the following formula:

$$MGT = \sum(Dn) / \sum n$$

Where,

D is the number of newly germinated seeds on each day and n is days of counting.

Ten seedlings emerged from the seeds were randomly sampled for determination of seedling growth at 10 days after sowing. The seedlings were separated from seed residuals (cotyledons) and seedling fresh weight was measured. Seedling dry weight was determined after the seedlings were dried at 70°C for 48 h in an oven.

The protocol of two seed vigor tests was described below.

Electrical conductivity test

Two replicates of 50 seeds from each pea cultivar were firstly weighed and then immersed in deionized water of 250 mL at 20°C for 24 h (ISTA 2003). After the incubation period, the electrical conductivity of soaked water was read by a conductivity meter. The results were divided to seed weight and it was expressed in µS cm^{-1} g^{-1} to evaluate the variability in seed weight among pea cultivars.

Controlled deterioration test

After the initial seed moisture content was determined, 50 g of seeds from each cultivar were placed into a plastic box with volume of 150 mL. Seed moisture content was increased to 20% by adding water. The amount of water requirement was calculated by the formula of ISTA (2003). Water volume (mL) = Seed weight (g) × [(100-Initial moisture content) / (100-Desired moisture content)]. Each box was tightly sealed and gently shaken three times during one hour and then the boxes were transferred to an incubator at 10°C for 24 h to allow slow and even imbibition by the seeds. They were then put into 45°C for a further 48 h in order to deteriorate the seeds. The standard germination test was subsequently conducted on the deteriorated seeds using 4 replicates with 50 seeds.

The experiment was established as a two factor completely randomized design (CRD). The chloride salts (NaCl, $CaCl_2$ and $MgCl_2$) were placed to first factor and the cultivars to second factor. Analysis of variance (ANOVA) was done by using the MSTAT-C computer software, version 2.10 (Michigan State University, v.2.10, USA). Significant differences among the means were compared by Duncan's Multiple Range test ($p<0.05$).

RESULTS AND DISCUSSION

Changes in seed moisture content, one hundred seed weight and protein content of eight pea cultivars showed in Table 1. Significant differences were determined among the pea cultivars for all parameters except for moisture content ($p<0.05$). The seed weight and protein content of pea cultivars were significantly different and the highest seed weight and protein content were recorded in Utrillo with 35.8 g and 26.8%, respectively.

Germination percentage, mean germination time, electrical conductivity and controlled deterioration test results of pea cultivars showed significant variation (Table 2). The lowest germination was counted in Electra (86.0%) and Utrillo (88.5%). The speed of germination was evaluated by mean germination time and it was significantly changed among the cultivars. The slowest germination was recorded in Utrillo with 5.10 days. Electrical conductivity and controlled deterioration tests suggested that the seed vigor of pea cultivars was considerably different from each other.

The EC values of the cultivars ranged from 19.2 to 60.6 µS cm^{-1} g^{-1} and the germination percentage after CD test changed between 0.0% and 93.0%. The higher electrical conductivity resulted in the lower germination after CD. These tests confirmed that Esprit and Puccet had the vigorous seeds while the weakest seeds were observed in Rainer.

Table 1: Initial seed characteristics of the investigated pea cultivars.

Cultivar	Moisture content (%)	Seed weight (g)	Protein content (%)
Ambassador	7.1	19.6^{d}	24.1^{b*}
Bolero	6.8	22.2^{b}	24.1^{b}
Electra	7.1	17.7^{ef}	22.2^{cd}
Esprit	7.1	19.1^{d}	21.9^{d}
Puccet	7.2	20.6^{c}	24.4^{b}
Rainer	6.9	16.9^{f}	23.6^{b}
Utrillo	7.2	35.8^{a}	26.8^{a}
Winner	6.9	18.1^{e}	23.3^{bc}
$LSD_{5\%}$	ns	0.87	1.34

*Means followed by the same letter(s) are not significantly different at $p<0.05$. ns: not significant.

Table 2: Germination and seed vigor performance of the investigated pea cultivars.

Cultivar	Germination (%)	Mean germination time (day)	EC (µS cm^{-1} g^{-1})	Germination (%) after CD
Ambassador	98.5^{a}	3.84^{b}	27.3^{d}	67.5^{c}
Bolero	98.5^{a}	4.03^{b}	26.7^{d}	80.0^{b}
Electra	86.0^{c}	3.85^{b}	41.2^{b}	16.0^{e}
Esprit	93.5^{b}	4.02^{b}	19.2^{e}	89.0^{a}
Puccet	98.0^{a}	4.02^{b}	22.2^{e}	93.0^{a}
Rainer	76.0^{d}	5.48^{a}	60.6^{a}	0.0^{f}
Utrillo	88.5^{c}	5.10^{a}	43.7^{b}	17.0^{e}
Winner	99.0^{a}	3.94^{b}	32.5^{c}	26.0^{d}
$LSD_{5\%}$	4.57	0.246	3.07	8.31

*Means followed by the same letter(s) are not significantly different at $p<0.05$.

Several significant correlations among the investigated parameters in eight pea cultivars were calculated. However, germination percentage under $CaCl_2$ and in $MgCl_2$ conditions were not associated with seed vigor tests (Table 3,4). Negative and significant correlation coefficients were determined for germination percentage in NaCl with EC and MGT, while germination percentage and CD test were related to NaCl. Also, germination percentage was negatively correlated with MGT and EC test. The EC and CD tests were only correlated with germination percentage under NaCl stress.

Germination and seedling growth changed with chloride salts. The sodium chloride salt has the least adverse effect, while magnesium chloride salt has more deleterious effect than the others. Similar results were reported by Abd El-Samad and Shaddad (2008) in pea. The chickpea genotypes also showed different responses to chloride salts and NaCl was the lowest hazardous salt among chloride salts (Özaktan *et al.* 2018). Our results showed that pea genotypes exhibited different responses to different salts. Among the cultivars, Winner was more sensitive to $CaCl_2$ and $MgCl_2$ while Utrillo was susceptible to $MgCl_2$. Yildirim *et al.* (2008) and Shadid *et al.* (2012) also found different sensitivity levels to salinity stresses during early growth stage.

The electrical conductivity test is the suitable seed vigor test for pea as suggested by ISTA (2003). The results of the present study showed that EC test successfully ranked the seed vigor of the pea genotypes. EC test showed that the most vigorous seeds were obtained from Esprit and Puccet. Our EC test results are in collaboration with the findings of Hampton *et al.* (2004), Panabianco *et al.* (2007) and Atak *et al.* (2008) who used the EC test to distinguish the pea seed lots as weak and vigorous seeds. Moreover, controlled deterioration test classified the seeds of pea cultivars in terms of seed vigor and its results confirmed the results of EC test; resulting in Esprit and Puccet were the most vigorous pea cultivars. We argued that the controlled deterioration test may be useful for pea seeds, but this test should be conducted with a lot of pea seed lots. However, germination and seedling performance of pea cultivars were different under chloride salt stresses, Bolero showed the superiority under $CaCl_2$, but Esprit and Bolero were better in $MgCl_2$ and NaCl conditions.

Table 3: Germination and seedling growth of pea cultivars subjected to distilled water and chloride salts of $CaCl_2$, $MgCl_2$ and NaCl solutions in equilibrium with 20 dS m^{-1}.

Cultivars	$CaCl_2$	$MgCl_2$	NaCl	Distilled water
Germination percentage (%)				
Ambassador	79.5	73.0	88.5	98.5^{a}
Bolero	89.0	76.0	98.0	98.5^{a}
Electra	70.5	72.5	74.0	86.0^{c}
Esprit	68.0	41.5	92.5	93.5^{b}
Puccet	95.0	57.0	97.0	98.0^{a}
Rainer	59.0	64.5	59.5	76.0^{d}
Utrillo	24.0	6.0	57.0	88.5^{c}
Winner	11.5	18.0	79.5	99.0^{a}
Mean germination time (day)				
Ambassador	6.43	8.26	5.31	3.84^{b}
Bolero	6.01	8.06	5.08	4.03^{b}
Electra	6.32	8.06	5.71	3.85^{b}
Esprit	6.18	8.09	5.62	4.02^{b}
Puccet	6.10	8.57	5.96	4.02^{b}
Rainer	5.94	7.45	5.19	5.48^{c}
Utrillo	-	-	7.61	5.10^{a}
Winner	-	-	5.31	3.94^{b}
Seedling fresh weight (mg $plant^{-1}$)				
Ambassador	32.4	36.5	63.0	89.5
Bolero	55.3	31.1	67.5	96.8
Electra	46.4	33.1	50.1	74.1
Esprit	38.3	30.4	72.4	83.7
Puccet	29.0	34.3	52.5	92.8
Rainer	24.7	30.9	38.5	54.5
Utrillo	19.0	-	38.0	54.4
Winner	-	-	15.4	31.9
Seedling dry weight (mg $plant^{-1}$)				
Ambassador	5.05	6.35	8.00	9.43
Bolero	8.45	7.45	11.55	15.82
Electra	6.15	6.35	7.05	8.90
Esprit	8.20	4.65	11.15	9.08
Puccet	5.05	6.15	10.45	11.58
Rainer	4.40	6.20	4.30	5.48
Utrillo	5.80	-	6.90	7.40
Winner	-	-	3.40	4.65

*Means followed by the same letter(s) are not significantly different at $p < 0.05$. Data could not be taken due to inadequate germination or no seedling growth.

Table 4: Pearson's correlation coefficients among seed vigor tests and germination percentage of pea under chloride salts.

	Germination	MGT	EC	CD	$CaCl_2$	$MgCl_2$
MGT	-0.775*	1				
EC	-0.885**	0.797*	1			
CD	0.729*	-0.606	-0.907**	1		
$CaCl_2$	0.158	-0.347	-0.372	0.649	1	
$MgCl_2$	-0.059	-0.285	-0.029	0.270	0.846**	1
NaCl	0.787*	-0.792*	-0.884**	0.923**	0.647	0.420

*: $p<0.05$, **: $p<0.01$ MGT: mean germination time, EC: electrical conductivity, CD: controlled deterioration.

The relationship between seed vigor tests and germination performance of pea cultivars under chloride salt conditions revealed that germination, MGT, EC and CD related with germination under NaCl stress. The highest significant and positive correlation was found in CD test, while the EC test gave negative and strong relationship under NaCl. The vigor tests did not significantly correlate with the germination under calcium and magnesium chloride stresses.

CONCLUSION

It was concluded that salinity tolerance in pea is primarily depended on genotypic factors; however, this study showed that seed vigor secondarily affected it. Pea genotypes showed different responses to chloride salts, but the cultivars with vigorous seeds showed better performance than the seeds with low vigor. For these reasons, it is strongly suggested that the newly harvested vigorous seeds must be used for production under salt infected areas to get uniform and sufficient plant emergence. Moreover, CD and EC tests' results should be only used for an indicator germination performance of pea under NaCl, further seed vigor tests should be searched for $MgCl_2$ and $CaCl_2$ stresses.

ACKNOWLEDGEMENT

The author thanks to Alfa Tohum Seed Company for supplying seeds samples of pea cultivars and to Prof.Dr. M.D. Kaya, Department of Field Crops, Eskişehir Osmangazi University for allowing his laboratory for this study.

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