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DARK GREEN COLORED SEEDS INCREASE THE SEED VIGOR AND GERMINATION ABILITY IN DRY GREEN PEA (*PISUM SATIVUM* L.)

MEHMET ATAK[1*], MEHMET DEMIR KAYA[2], GAMZE KAYA[2], MUHARREM KAYA[3] AND KHALID MAHMOOD KHAWAR[4]

[1]Department of Field Crops, Faculty of Agriculture, Mustafa Kemal Univesity, Hatay- Turkey,
[2]Central Field Crops Research, Ministry of Agriculture and Rural Affairs, Yeni Mahalle, Ankara, Turkey,
[3]Department of Field Crops, Faculty of Agriculture, Suleyman Demirel University, Isparta, Turkey,
[4]Department of Field Crops, Faculty of Agriculture, University of Ankara, Turkey.
[*]Corresponding: E-mail: kmkhawar@gmail.com Tel: 0090 312 596 15 39, Fax: 0090 312 3182666

Abstract

Three green-seeded dry pea genotypes cvs. 'Rondo', 'Carina' and 'Jof' with each genotypes producing a mixture of light (L), medium (M) and dark (D) green seed at maturity were evaluated for their germination behavior and seedling growth for salinity tolerance. Electrical conductivities of NaCl solutions were 0.0, 5.0, 10.0, 15.0, or 20.0 dS m^{-1}. The objective of this study was to determine the influence of green pea seed coat color from light to dark green on germination ability and seedling growth under varying salinity conditions. The results revealed that increased salinity levels generally resulted in decreased measurement of all the traits but mean germination time was increased with higher salinity levels. Of the three genotypes, cv. Carina had the highest germination percentage, shoot and root length, shoot fresh weight, root fresh weight, and the fastest mean germination time. Dark green colored seeds germinated faster than light ones. Additionally, the dark green colored seeds had the highest shoot and root length and fresh weight. The accelerated ageing (AA) and the electrical conductivity (EC) tests showed significant differences in seed vigor of cultivars and green color tones ($p<0.01$). The results of AA and EC tests confirmed that cv. Carina was superior to the others in terms of seed vigor. Additionally, the dark green seeds of the cultivars showed higher seed vigor than that of light and medium. Our results suggest that cultivars with dark green colored seeds could be preferred on saline conditions due to their high seed vigor and seedling growth ability.

Iintroduction

Pea (*Pisum sativum* L.), a cool season food legume, is broadly grown in Turkey and other countries, as its seed is a low-cost source of protein. Pea cultivation is widespread in areas having a mild and warm climate, because relatively high or low temperatures are the most important factors limiting pea cultivation. A dry climate is also unsuitable for the plant, particularly during flowering and pod development (Bozoglu *et al*., 2007). Heterogeneity among seeds is mostly attributed to various genetic, physiological and environmental stimulants, which could cause variations in size, color, weight and shape of seed even in the same cultivar. As in most legumes, single individual pea plant or pod produces seeds differing in their appearance. In grain legumes, sequential development and spatial heterogeneity of the pod position can lead to significant differences in shape, size, weight and color among the seeds (Fenner, 1993; Coste *et al*., 2001; 2005). Such differences may affect the subsequent physiological properties (e.g., germination and dormancy) of the seeds (Kahn *et al*., 1996). Improved seed germination may warrant a better seedling formation following successful stand establishment and hence, crop

production (Almansouri *et al*., 2001; Sadeghian & Yavari, 2004). Crop establishment depends on an interaction between seedbed environment and seed quality (Khajeh-Hosseini *et al*., 2003). One of the environmental factors adversely affecting seed germination is sensitivity to salinity stress (Sadeghian & Yavari, 2004). Vigorous seeds are usually expected to recover quicker from such stress factors. The seed vigor tests will ensure a better seed and stand establishment quality and various vigor tests may provide different results for different genotypes (Powell *et al*., 1997; Jotai *et al*., 2001; Hampton *et al*., 2004). Vigor tests are desirable in that they present coherent results with field seedling emergence. The accelerated ageing (AA) test holds seeds at a high temperature and high humidity for a specified time and helps predict the field emergence of many species (Kulik & Yaklish, 1982; Anon., 1995). The AA method has been particularly suggested for pea, since better seedling vigor was obtained in pea seeds which had higher AA test values (Hampton *et al*., 2004).

The electrical conductivity (EC) test also proved for the selection of highly vigorous pea seed lots in sowing at unfavorable stress conditions (Matthews & Powell, 1981). A positive relationship was found between EC measurements and germination capacity of pea seeds (Anon., 1995; Taweekul *et al*., 1998; Vieira *et al*., 1999; Siddique *et al*., 2002).

Although the crop is ranked among the salt sensitive crops like other leguminous crops and produce low yield even at mild salt stress (Francois & Mass, 1994), a detailed information on genetic variability for salt tolerance is still lacking in the literature (Noreen *et al*., 2007). The objective of the present study was to determine the influence of green pea seed color (from light to dark green) on germination behavior and seedling growth in increasing salinity conditions. The long-term agronomic purpose is to improve the establishment of pea cultures in arid regions. So far no reports have been published about the effects of seed color tones on seed vigor.

Materials and Methods

Seed materials and test solutions: The seeds of wrinkled pea genotypes of cv. Rondo, Carina and Jof were provided by Variety Registration and Seed Certification Institute of Turkey. All the seeds were stored at 4^{o}C temperatures to maintain same moisture content until the start of the experiment. In pea, the three color tones were previously reported as green, yellow-green and yellow (Borowska *et al*., 1998). For this study, dark (D), medium (M) and light (L) coat colors were used to avoid any confusion since the genotypes includes only green colored seeds. Seed colors were determined by visual inspection of the seed coat color. Salt concentrations of the solutions with the electrical conductivities of 5.0, 10.0, 15.0, or 20.0 dS m^{-1} were adjusted using NaCl, with distilled water served as a control.

Germination test: Three replicates of 50 seeds from each cultivar and seed color were germinated in 3 rolled filter papers with 10 mL of respective test solutions. The papers were replaced every 2 days to prevent subsequent salt accumulation (Rehman *et al*., 1996). In order to prevent evaporation, each rolled paper was placed into a sealed plastic bag. Seeds were allowed to germinate at 20 ± 2^{o}C in the dark for 10 days in a growth chamber. Seeds were considered germinated when the emerging radicle was at least 2 mm long (Murillo-Amador *et al*., 2002). Germination percentage (GP) was recorded

every 24 h for 10 days. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis & Roberts, 1980) as follows:

$$MGT = \Sigma(fx) / \Sigma f$$

where f is the number of newly germinated seeds on each day and x is the day of counting. Root length (RL), shoot length (SL), shoot fresh weight (SFW) and root fresh weight (RFW) were measured on the 10^{th} day. The seedling with short, thick and spiral formed epicotyls and stunted primary roots were considered as abnormally germinated and discarded from further analyses.

Accelerated ageing (AA) test: Four replicates of 50 g seed were sampled from the each genotype and seed color. The AA test was performed by using an ageing temperature and time combination of 42°C for 48 h in a dark growth chamber (Hampton *et al*., 2004). After ageing, 25 seeds per replicate were germinated using filter paper at 20 ± 2°C in dark growth chamber for 10 days. Germination percentages (GP) were recorded on 10^{th} day.

Electrical conductivity (EC) test: The EC test for seed vigor was performed with the genotypes and color tones. Two replicates of 50 seeds from each genotype and seed color were weighed and then immersed in 250 mL deionized water at 20°C for 24 h (Anon., 1995; Powell *et al*., 1997). The electrical conductivity of soaked water was measured using a conductivity meter (Model WTW Cond 314i, Germany). Results were expressed in µS cm^{-1} g^{-1} to take account of variability in seed weight among the seeds lots.

Statistical analysis: The experimental design was three factors factorial (5 x 3 x 3) arranged in randomized complete block design with 3 replications and each experimental unit contained 50 seeds. The first factor was NaCl solutions (0.0, 5.0, 10.0, 15.0 and 20.0 dS m^{-1}), the second was genotypes (Rondo, Carina and Jof), and the third was seed colors (light, medium and dark green). For AA and EC tests, two factors factorial (3 x 3) was arranged in completely randomized design with four replicates. The first factor was genotypes and the second was seed colors. Data for germination percentage were subjected to arcsine transformation before statistical analysis. Analysis of variance was performed using the SAS software package (Anon., 1998). Significant differences among the mean values were compared by LSD test ($p< 0.05$).

Results

For all the germination and seedling characteristics, the main effects, two and three-way interaction mean squares with their significance levels were displayed on the ANOVA (Table 1). The results of AA and EC tests was also mentioned in the text.

Germination and seedling growth: A significant two-way interaction (genotype x seed color) was found for GP ($p<0.05$). The darker seed color resulted in the higher germination percentage (Table 2). Genotypes with dark seed lead to a higher GP at all NaCl conditions. Dark seeds of cv. Rondo, Carina and Jof possessed higher GP values of 85.0, 100.0 and 100.0 % at 20 dS m^{-1}, respectively. When the salinity levels increased,

GP levels decreased accordingly. Among the three genotypes, the lowest GP reduction due to salinity was determined in cv. Carina (Table 2).

Mean germination time (MGT) was significantly longer than others at 20 dS m^{-1} (Table 3). Increased NaCl levels caused a remarkable increase in MGT. The earliest germination was observed in Jof. However, the dark seeds shortened the germination time indicating that germination was improved as the seed was greener. At the highest salinity level, the shortest MGT (2.49 d) was observed in Jof, which also had the shortest MGT at control treatment (Table 3).

Table 1. Analysis of variance for the mean squares of germination percentage (GP), mean germination time (MGT), shoot length (SL), root length (RL), shoot fresh weight (SFW) and root fresh weight (RFW)of pea genotypes as affected by salinity, genotype and color.

Sources	df	GP	MGT	Sources	df	SL	RL	SFW	RFW
Blocks	2	19.3	0.04	Blocks	2	0.009	0.11	175.2	148.7
Salinity (S)	4	233.5*	1.99**	Salinty (S)	4	45.3**	65.3**	92554.1**	41024.8**
Genotype (G)	2	7244.2**	9.6**	Genotype (G)	2	63.4**	79.9**	68248.0**	35554.9**
Color (C)	2	768.8**	0.25*	Color (C)	2	8.8**	15.7**	18062.4**	11947.9**
S x G	8	26.7 ns	0.22**	S x G	6	1.1*	4.16**	1987.0ns	901.2 ns
S x C	8	83.7 ns	0.08ns	S x C	8	1.15**	1.7*	3178.8**	1013.4ns
G x C	4	206.9*	0.03ns	G xC	4	3.13**	7.2**	3608.9**	73.59 **
S x G x C	16	73.9 ns	0.05ns	S x G x C	12	0.75 ns	2.7**	2836.9**	1862.2**
Error	88	69.8	0.05	Error	76	0.412	0.76	907.1	534.4
Total	**134**	-	-	**Total**	**116**				

** = Significant at p<0.01, *= Significant at p<0.05, ns, non-significant

Table 2. Germination percentage (GP) of pea genotypes as affected by salinity and seed color.

Salinity (S)	GP (%)*									
	Rondo			Carina			Jof			
	L	M	D	L	M	D	L	M	D	Mean (S)
Control	76.6	78.0	96.6	100.0	100.0	100.0	98.3	100.0	100.0	94.4
5 dS m^{-1}	75.3	83.0	87.0	100.0	100.0	100.0	100.0	96.6	100.0	93.6
10 dS m^{-1}	65.0	90.6	91.3	98.3	100.0	100.0	96.0	100.0	98.0	93.3
15 dS m^{-1}	66.6	78.0	87.6	96.6	100.0	100.0	93.3	98.0	95.0	90.6
20 dS m^{-1}	61.3	65.6	85.0	98.3	96.6	100.0	92.6	98.3	100.0	88.3
$LSD_{0.05}$	G x C =6.063			S=4.519 (88df)						

*Values show the real germination percentages but variance analysis was performed using arcsine transformed values

Table 3. Mean germination time (MGT) of pea genotypes affected by salinity and seed color.

Salinity (S)	MGT (d)								
	Rondo			Carina			Jof		
	L	M	D	L	M	D	L	M	D
Control	3.25	2.84	2.64	2.26	2.14	2.15	2.09	1.64	1.94
5 dS m^{-1}	3.13	3.03	2.73	2.31	2.23	2.21	2.05	2.08	2.10
10 dS m^{-1}	2.80	2.89	2.88	2.25	2.28	2.28	2.22	2.12	2.22
15 dS m^{-1}	2.84	2.87	2.92	2.33	2.34	2.26	2.39	2.49	2.26
20 dS m^{-1}	4.03	3.47	3.85	2.84	2.82	2.60	2.69	2.45	2.41
Mean (C)	**2.64 (Light)**			**2.52 (Medium)**			**2.49 (Dark)**		
$LSD_{0.05}$	S x G=0.209			C=1.162 (88 df)					

Analysis of variance showed that shoot length was significantly affected by different salinity levels, seed color tones or the pea cultivars used in this study (p<0.01). Shoot length (SL) gradually decreased as salinity levels increased (Table 4). The results of two-

way interaction (salinity x genotype) showed that higher concentration of NaCl than 10 dS m^{-1} caused no shoot formation in cv. Rondo (Table 4). Of the three genotypes and seed colors, the dark seeded cv. Carina had the longest shoots with 4.84 cm (Table 4).

Root length (RL) was adversely influenced by NaCl, but growth inhibition was the greatest in cv. Rondo. None of the seedlings of cv. Rondo was able to grow into a plant at 15 dS m^{-1} or above (Table 4). Dark seeds of genotypes showed longer root length under all stress conditions while 5 dS m^{-1} promoted root growth for light seeds of both cv. Rondo and Jof (Table 4).

Table 4. Shoot length (SL) and root length (RL) of pea genotypes as affected by salinity and seed color.

Salinity (S)	Rondo			Carina			Jof		
	L	M	D	L	M	D	L	M	D
	SL (cm)								
Control	1.76	2.62	4.31	5.39	6.07	8.16	4.78	6.28	5.26
5 dS m^{-1}	2.10	2.60	3.15	4.86	4.97	6.66	4.88	4.37	4.12
10 dS m^{-1}	1.95	1.17	2.69	4.08	4.12	4.78	4.27	4.49	3.70
15 dS m^{-1}	-	-	-	2.65	3.95	3.48	2.68	3.11	3.40
20 dS m^{-1}	-	-	-	1.42	1.59	2.85	2.00	1.52	2.08
$LSD_{0.05}$	S x G= 0.602,			S x C=0.602,			G x C=0.466 (76 df)		
	RL (cm)								
Control	3.62	4.83	6.66	8.26	6.55	11.31	5.60	7.56	7.27
5 dS m^{-1}	4.16	4.80	6.36	7.84	8.80	11.26	7.10	5.66	4.80
10 dS m^{-1}	4.56	4.16	5.46	6.06	6.90	8.80	6.53	5.70	6.03
15 dS m^{-1}	-	-	-	5.03	5.33	5.76	5.13	4.50	4.26
20 dS m^{-1}	-	-	-	3.33	3.43	4.16	3.16	2.16	3.26
$LSD_{0.05}$	S x G x C=10.418 (76 df)								

Table 5. Shoot fresh weight (SFW) and root fresh weight (RFW of pea genotypes affected by salinity and seed color.

Salinity (S)	Rondo			Carina			Jof		
	L	M	D	L	M	D	L	M	D
	SFW (mg $plant^{-1}$)								
Control	105.6	169.5	260.6	259.8	254.7	353.7	229.1	276.9	237.1
5 dS m^{-1}	129.3	143.3	159.9	211.6	227.3	281.4	225.8	193.0	235.3
10 dS m^{-1}	127.8	93.0	149.4	203.1	189.5	215.9	195.0	202.8	156.0
15 dS m^{-1}	-	-	-	123.0	126.5	132.5	131.5	137.9	131.0
20 dS m^{-1}	-	-	-	74.7	80.1	103.0	86.3	78.1	154.3
$LSD_{0.05}$	S x G x C=48.98 (76 df)								
	RFW (mg $plant^{-1}$)								
Control	75.5	97.4	163.9	142.3	130.3	245.5	141.8	213.9	162.5
5 dS m^{-1}	98.7	106.7	136.8	152.9	186.1	263.3	194.7	179.0	137.9
10 dS m^{-1}	100.9	75.3	112.4	125.6	136.3	174.5	155.0	162.1	148.5
15 dS m^{-1}	-	-	-	91.7	114.7	134.3	93.8	97.1	94.3
20 dS m^{-1}	-	-	-	53.7	74.5	97.5	75.5	52.7	79.9
$LSD_{0.05}$	S x G x C=37.59 (76 df)								

A significant variation in shoot fresh weight (SFW) was observed in cv. Rondo under increased NaCl conditions ($p<0.01$). No shoot fresh weight was recorded at and over 15 dS m^{-1} of NaCl (Table 5). Along with decreasing shoot length, shoot fresh weight seemed to be slowly declined with the increasing NaCl. However**,** shoot growth was significantly improved in dark seeds at all NaCl levels ($p<0.01$). Cv. Carina had the largest SFW of the three genotypes and the dark seeded cv. Carina had the highest SFW (Table 5).

Salinity x genotype x seed color interaction was found significant ($p<0.01$) for root fresh weight (RFW). The highest RFW was detected in dark colored seeds of cv. Carina at 5 dS m^{-1} (263.3 mg $plant^{-1}$; Table 5). In highest salinity conditions dark green seeded cv. Carina had the higher RFW values (Table 5).

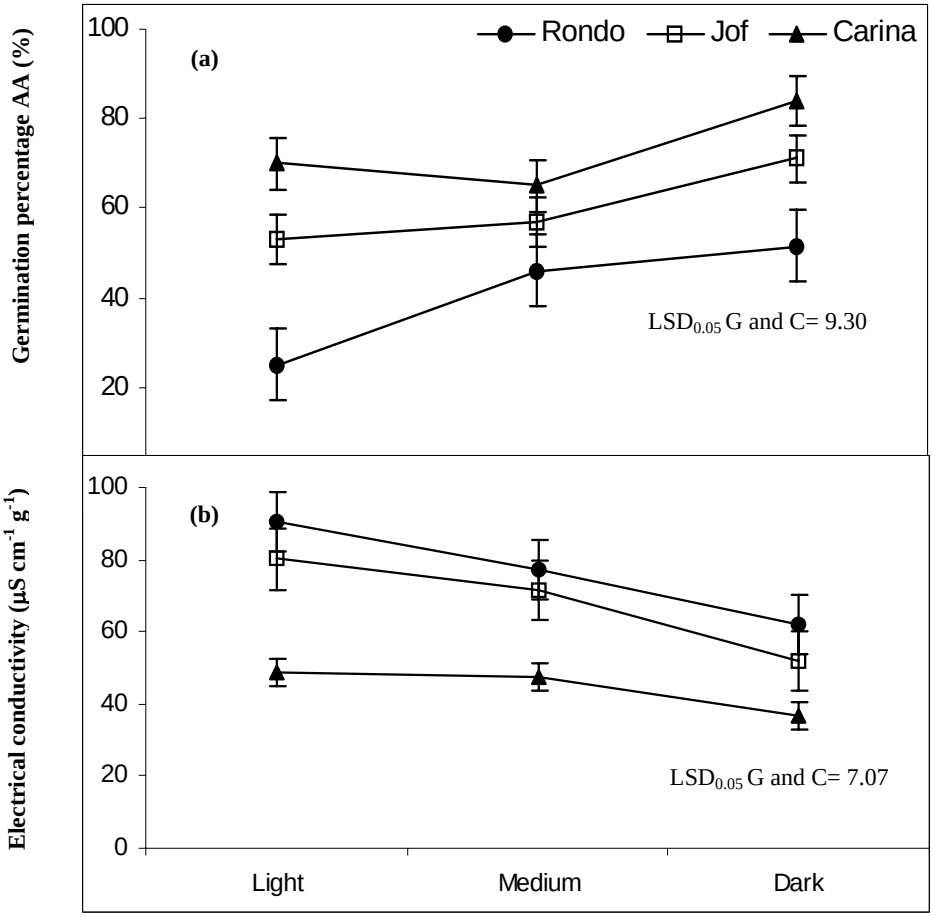

Fig. 1. Changes in germination percentage after accelerated ageing (a), and electrical conductivity results of pea genotypes in relation to seed colors tones (b).

Seed vigor (AA and EC) tests: The GP values in AA test were dissimilar to that of previous germination test as only main effects were significantly different ($p<0.01$). The light and medium green seeds showed the lowest GP, regardless of genotypes (Fig. 1a).

As for genotypes averaged over seed colors, cv. Carina had the higher GP compared to GP of cv. Carina and Jof (Fig. 1a). Evidently, cv. Carina seemed to have a better seed vigor than the others. In terms of seed colors, the dark seeds of the genotypes had also the highest GP. As expected, EC values steadily declined when the seeds of the genotypes were darkened (Fig. 1b) and cv. Carina had the lowest EC values. The test results of AA appeared in corroboration with EC test.

Discussion

Increased NaCl concentrations and seed coat color tones influenced the germination and seedling growth of the different pea genotypes. The NaCl solutions in the growth conditions had greater inhibitory effect on GP and MGT which were also considerably affected by seed colors from light to dark green. Tolerance to salinity increased as the green color was darker. Cerda *et al*., (1982) and Okcu *et al*., (2005) found distinct genetic variation among pea cultivars for germination and seedling growth subjected to increased NaCl concentration. Similar findings were reported by Murillo-Amador *et al*., (2002) in cowpea. Okcu *et al*., (2005) also reported that the pea genotypes were able to germinate at EC values up to 16.3 dS m^{-1} without a significant decrease in GP. However, no experiments have been reported that the seed color differences cause variation in germination properties of a particular green pea genotype. In our experiment, the dark seeds of cv. Carina were able to germinate at as high as 20 dS m^{-1} salinity level without any significant decrease in GP but a significant increase in MGT.

Shoot and root lengths as well as fresh weights also improved both GP and MGT in dark green seeds even in higher saline conditions. Increased salt concentrations were reported to decrease shoot and root lengths and fresh weights of pea (Hasson-Porath *et al*., 1972; Okcu et al., 2005) as well as other cereal seedlings (Salim, 1991; Atak *et al*., 2006). This might be because the presence of NaCl in the germination medium reduces the uptake of water by the seedlings, and inhibits the mobilization of the cotyledon reserves to the growing embryonic axis (Gomes-Filho & Prisco, 1978; Gomes Filho *et al*., 1983). These findings are in agreement with others that involved germination studies in the presence of NaCl or nonionic osmotic solutions such as mannitol or polyethylene glycol (Gomes Filho & Sodek, 1988; Murillo-Amador *et al*., 2002; Kaya *et al*., 2006). Our findings also revealed that dark green seed of one genotype may be superior or inferior to other dark green seeded genotypes in terms of the germination and other seedling characteristics of pea. For example, dark green colored cv. Carina seeds had advantages in terms of GP and other seedling characteristics as compared to other dark seeded genotypes. Therefore, the variation in genotypes and/or seed color may be taken into account when selecting suitable cultivars with better seed vigor and stand establishments even in highly saline environments.

Differences in seed vigor among seed colors and genotypes were determined by vigor tests (AA and EC tests). Although no two-way interactions for genotype x seed color obtained, significant differences were obtained among cultivars and among seed colors. Cv. Rondo, having the least GP at 20 dS m^{-1}, had the lowest GP values after AA and the highest EC value in soaked water (Fig. 1a, b). The finding of this study are consistent with Jotai *et al*., (2001) and Hampton *et al*., (2004), who observed significant cultivar differences in AA tests in pea. The dark seeds had the shortest MGT values indicating a better seed vigor than medium and light colored seeds. This result was consisted with previous study (Khan *et al*.,

2003) which showed that high vigor pea seeds had low level of leakage into soak water and hence low electrical conductivity. High leakage of low vigor seeds was attributable to dead tissue and the cotyledons and low conductivity represents the higher seed vigor (Powell *et al*., 1997). Furthermore, a positive relationship was found between EC measurements and germination capacity of pea seeds in field performance by Taweekul *et al*., (1998) and Siddique *et al*., (2002). Kolasinska *et al*., (2000) reported a strong relationship between EC test results and field emergence rate of bean. In our study, EC test revealed that cv. Carina and dark seed color seemed to have high seed vigor. Our vigor test results are in corroboration with the findings of the results from germination test.

In legumes, heterogeneity within a seed population could result in variation in germination ability (Matilda *et al*., 2005). The appearance of uniform green pea color is not only important for a better stand establishment even in unfavorable environmental conditions but also the most important quality criteria in domestic and international markets. The results revealed that seed color tones are also important for seed quality. Since color differences may affect the seedling vigor even in saline conditions, this information can be successfully used when choosing better seeds. Color sensors or a portable spectrophotometer (Coste *et al*., 2005) may aid separation of the dark seeds and making it easier to grow peas in large acreages. In addition, selecting the pods matured at the same time can be advantageous in terms of obtaining same dark green colored seeds which can provide better seedling vigor even in saline conditions.

Seed color heterogeneity within a pea genotype is mostly attributable to either chlorophyll disappearance or bleaching (McCallum, 1997; Cheng *et al*., 2004). Chlorophyll disappearance may occur during growth and development, harvest and storage (Fenner, 1985; Cheng *et al*., 2004). However, bleaching mostly occur due to adverse environmental conditions during maturation and was proven to be major cause of reduced seed vigor and germination (Fenner, 1985). Therefore, the seed color heterogeneity was most likely caused by the unfavorable environmental conditions during harvest not by the chlorophyll disappearance.

Our results showed that dark seeds performed better than light and medium green seeds even in saline conditions independent of varieties. In commercial production of peas under saline conditions, dark green-colored seed of cultivars and/or cultivars having the least variation in seed color could be recommended to farmers to attain higher GP and uniform emergence under field environment. In the present study, we visually separated seeds with respect to color tones, however, it would be much informative to use devices which color code the seeds and make separation with color sensors. In addition, the present study was carried out to determine the seed vigor of colored green pea seeds (from light to dark green) in controlled environments, however, future experiments in field conditions will give more conclusive results.

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