Pak. J. Bot., 36(2): 311-320, 2004.

RELATIONSHIPS AMONG SOME SEED TRAITS, LABORATORY GERMINATION AND FIELD EMERGENCE IN COWPEA (*VIGNA UNGUICULATA* (L.) WALP.) GENOTYPES

AYSUN PEKŞEN*, ERKUT PEKŞEN AND HATİCE BOZOĞLU

Faculty of Agriculture, Ondokuz Mayıs University, 55139 Samsun, Turkey
**Corresponding author e-mail: aysunp@omu.edu.tr*

Abstract

A study was carried out to investigate the relationships among percentage water absorption during imbibition, leaching of electrolytes from seeds into steep water, germination and field emergence of 21 cowpea (*Vigna unguiculata* (L.) Walp.) genotypes differing in testa colour. Water absorption rate of cowpea seeds during imbibition and electrical conductivity (EC) of steep water were determined at 1, 2, 3, 4, 5, 6, 12, 18 and 24 hours. Significant differences were found between white and coloured cowpea seeds in terms of water absorption rate depending on imbibition time. Amount of water absorbed by seeds exceeded initial seed weight at 3 hours in white seeded and 18 hours in coloured seeded genotypes. EC of steep water showed similar trend to water uptake of seeds in both white and coloured seeded genotypes. Water uptake significantly influenced germination and field emergence in white seeded genotypes. The effect of water absorption rate on germination and field emergence at the end of the soaking period was not significant in coloured seeded genotypes. Negative and significant correlation was found between electrical conductivity and field emergence in white seeded genotypes but this relationship was not significant in coloured genotypes. The present study showed that EC is an important test to predict field emergence potential of white seeded cowpea genotypes under field conditions.

Introduction

Quality seeds of improved varieties are the key to agricultural progress. The production potential and other desirable characteristics of seeds set the limits on production. Other inputs such as fertilizers, pesticides, herbicides and overall crop management also help to realize the production potential of seeds. Part of the success of a farmer's crop depends on the quality of seed (Srivastava, 1986). Seed testing is often the first step in enhancing the quality of the seed. The testing can be minimal only for germination, or very extensive for moisture, purity, germination, health and other characteristics (Gastel, 1986).

Some tests such as tetrazolium, seedling growth rate and seedling dry weight, accelerated aging test, first count, speed of germination, cool germination test and cold test are used to estimate seed vigour (Agrawal, 1986). A general rule is that high germination capacity is associated with high vigour. Seed vigour may be reduced by damage to the embryo or seed coat during harvesting and processing. Discrepancies between germination capacity and field performance are not equal in different species. In pulses, for instance, discrepancies occur much more commonly than in cereals (Burg, 1986). Standard laboratory germination test is the most common and practical seed vigour test, however, it may be insufficient in the determination of seed vigour.

Electrical conductivity (EC) is another test to evaluate seed vigour. It was developed into a routine vigour test by determining leakage of electrolytes from seeds into soaking water for the prediction of garden pea (*Pisum sativum* L.) field emergence (Matthews & Bradnock, 1967). This test also termed as the bulk conductivity test, is now extensively used in Europe, Australia and New Zealand for this species (Hampton & Coolbear, 1990). Rapidity of water uptake by seeds is an important cause of seed loss viability in legumes (Sivritepe & Dourado, 1995; Demir, 1996). Rapid water absorption in grain legumes including field bean may be the cause of poor emergence and seedling growth (Powell *et al*., 1984; Abdullah *et al*., 1991). The cream/beige cultivars show more evidence of imbibition damage as revealed by poor vital staining and high solute leakage (Legesse & Powell, 1992). Testa whiteness is also associated with poor emergence (Powell *et al*., 1986).

The objective of the present study was to determine the relationships among percentage water absorption during imbibition, the leaching of electrolytes from seeds into steep water, germination and field emergence of 21 cowpea (*Vigna unguiculata* (L.) Walp.) genotypes differing in testa colour.

Materials and Methods

Eighteen local cowpea genotypes collected from Agean and Mediterranean Regions of Turkey and 3 genotypes introduced from Japan, Azerbaijan and Taiwan were used in this study. The names of cowpea genotypes, their sources, testa and hilum colours, seed shape (Pekşen *et al*., 2000) and initial seed moisture contents are given in Table 1. The testa colour of G23, G26 and GK was claret red while G10 and G18 had black and mustard testa colour, respectively. The seed coat surface of coloured genotypes was smooth. All of the other cowpea genotypes had white and wrinkled testa.

Seed coats were removed from cotyledons by hand peeling after soaking in distilled water for 6 hours to determine the seed coat ratios. Seed coat and cotyledons were dried at 80°C for 24 hours and weighed separately. Then, seed coat dry weight was rated to total weight of seed coat and cotyledons to calculate seed coat ratio.

Cowpea seeds were steeped in 250 ml of distilled water for 24 hours at 20 °C. EC was determined using 3 replicates of 25 weighed dry seeds with a digital electrical conductivity meter. The exudation of electrolytes from each sample was measured after 1, 2, 3, 4, 5, 6, 12, 18 and 24 hours of soaking. Seeds were not removed from soaked water before reading. EC was expressed on the basis of 1 g initial seed weight before soaking. Changes in water absorption rate (WAR) of cowpea seed lots were also determined at the same time periods using four replicates of 25 seeds. Seeds were removed from soaking water and surface-dried between paper towel before weighing at each time periods.

The laboratory germination (LG) percentage and 1000 seed weight were determined by using method described by ISTA (Anon., 1985a, b). Germination was evaluated as the percentage of seed producing a 22 mm radicle. The field emergence (FE) was tested for three replicates of 100 seeds in a randomized complete block design. Seeds were sown in 3 cm soil depth on 26 June, 1999 in Samsun. The emergence criterion was the appearance of hypocotyl on the surface of the soil. Field soil was clay-loam, medium in organic matter, rich in potassium and poor in phosphorus and without lime. Soil reaction was slightly acidic. The experimental area was irrigated every few days. Seedling counts were determined at 2 day intervals from day 7^{th} and final FE was assessed at the end of 21 days.

Table 1. The names, sources and some seed characteristics of cowpea genotypes used in the study* (Peksen *et al*., 2000).

Cowpea genotypes	Sources of genotypes	Testa colour	Hilum colour	Seed shape	Initial seed moisture content (%)**
G1	Isparta	White	Black	Wrinkled	8.92
G3	Söke/Aydın	White	Black	Wrinkled	9.04
G4	Aydın	White	Black	Wrinkled	9.41
G5	Aydın	White	Black	Wrinkled	8.79
G6	İzmir	White	Light	Wrinkled	8.94
G7	Aydın	White	Brown	Wrinkled	9.23
G9	Turgutlu/Man	White	Black	Wrinkled	8.71
G10	**Manisa**	**Black**	**White**	**Smooth**	**8.75**
G14-A	Söke/Aydın	White	Brown	Wrinkled	9.48
G14-B	Söke/Aydın	White	Black	Wrinkled	9.46
G15-A	Eşme/Uşak	White	Brown	Wrinkled	8.51
G15-B	Eşme/Uşak	White	Black	Wrinkled	8.90
G16-A	Eşme/Uşak	White	Brown	Wrinkled	8.35
G16-B	Eşme/Uşak	White	Black	Wrinkled	8.98
G18	**Turgutlu/Ma**	**Mustard**	**Black**	**Smooth**	**9.53**
G19	Balıkesir	White	Black	Wrinkled	9.01
G20	Antalya	White	Brown	Wrinkled	9.68
G23	**Taiwan**	**Claret**	**White**	**Smooth**	**9.43**
G26	**Japonya**	**Claret**	**White**	**Smooth**	**9.61**
GA	Azerbeycan	White	Black	Wrinkled	9.31
GK	**Turgutlu**	**Claret**	**Black**	**Smooth**	**8.65**

*Coloured seeded genotypes are indicated with bold letters

** Determined by using high constant oven method (Anon., 1985a, 1985b)

Results and Discussion

Highly significant differences ($P<0.01$) were found among cowpea genotypes in terms of 1000 seed weight and seed coat ratio. Generally, 1000 seed weight of coloured genotypes except for G10 genotype was lower than all of the white seeded genotypes except for G20. The highest 1000 seed weight of 296.50 g was observed in black seeded G10 genotype (Fig.1).

Coloured seeded genotypes, shown as a separate group on the right side in Fig. 1, had two times higher seed coat ratios than white seeded genotypes. Seed coat ratios of coloured seeds ranged from 10.32 % in G18 to 11.57 % in G26, while this ratio varied from 4.81 % in GA to 7.70 % in G14-A for white seeded ones. The highest seed coat ratios were determined in G26 (11.57%) and G23 (11.54%) genotypes (Fig. 1).

Water absorption rate (WAR): Initial seed moisture contents of cowpea genotypes varied between 8.35% and 9.68% (Table 1). Analysis of variance confirmed that the differences among cowpea genotypes for WAR were significant ($P<0.01$). WAR of coloured cowpea genotypes ranged from 105.56% in G23 to 124.04% in G10, while WAR of white seeded genotypes varied between 107.82% in G3 and 123.64% in G9 at the end of the 24th hour (Fig. 2 and 3).

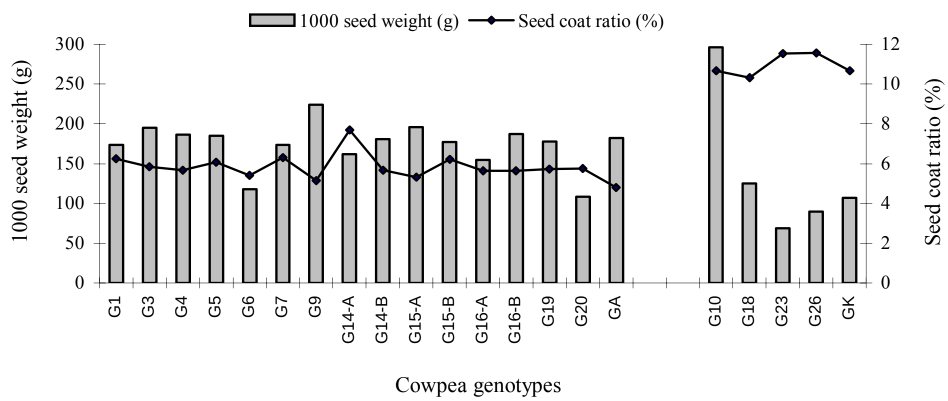

Fig. 1. 1000 seed weight and seed coat ratio of cowpea genotypes used in the study.

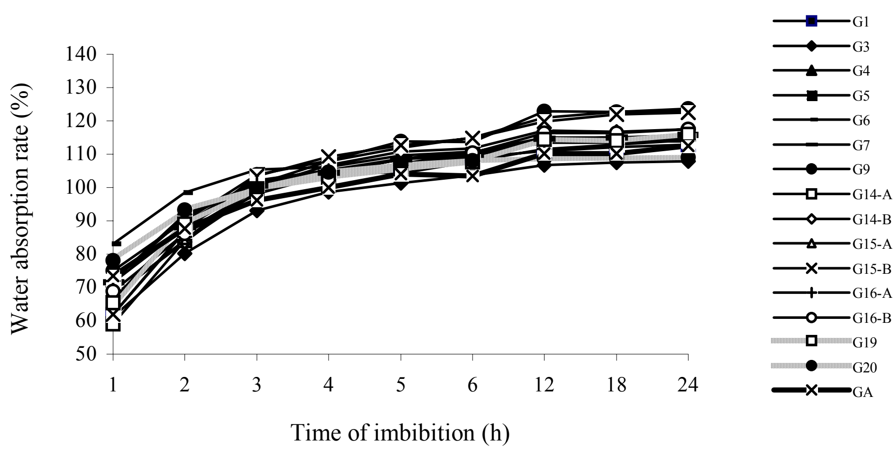

Fig. 2. Changes in water absorption rate during the course of imbibition time of cowpea genotypes having white seed coat.

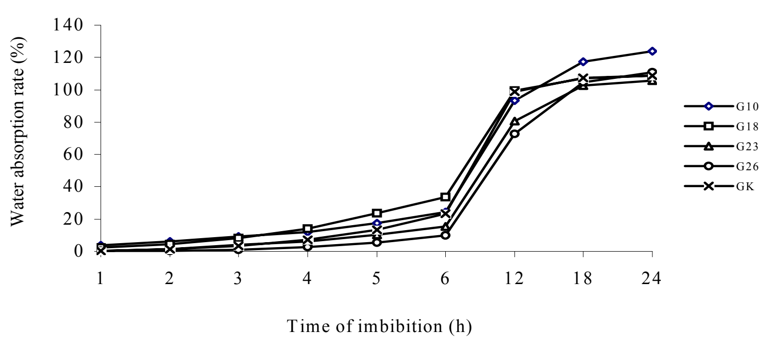

Fig. 3. Changes in water absorption rate during the course of imbibition time of cowpea genotypes having coloured seed coat.

Evident differences were found between white and coloured cowpea seeds in terms of WAR depending on imbibition time. Rapid water absorption occurred in white and wrinkled seeded cowpea genotypes during the first three hours of imbibition when compared to coloured seeds. It may be attributed to more permeable testa adhered loosely to cotyledons in white seeded genotypes and their low seed coat ratio. Water uptake of white seeded cowpea genotypes slowed down again from 3 to 6 hours and there was no significant increase in water absorption after 12 hours of imbibition (Fig. 2).

The change with time in water absorption rate of coloured seeds displayed a sigmoid curve (Fig. 3 and 6) similar to reports of Yoshida *et al*., (1995) in *Vigna angularis* cv. Erimo-shozu. Very slow water absorption occurred in genotypes having coloured and smooth seed coat up to 6 hours. WAR of coloured seeds evidently increased from 6 to 12 hours by increasing in permeability of testa and slowed down again after 12 hours. There was no significant changes in water uptake of coloured seeds after 18 hours. The amount of water absorbed by white and coloured seeds exceeded their initial seed dry weight after 3 hours (Fig. 2) and 18 hours (Fig. 3), respectively.

Cowpea cultivars with completely or partially cream/beige testas imbibed more rapidly than cultivars with coloured testas. Water uptake by the slowly imbibing coloured cultivars are limited by the testa since embryos imbibed rapidly. The permeability of the testa is the major factor contributing to the slow imbibition of coloured seeds although water uptake is also restricted by the micropyle (Legesse & Powell, 1992). Similar results were found for WAR in the present study. Slow water absorption of coloured seeds may have sourced from coloured testa which adhered close to cotyledons or pigmentation of the seed coat during maturation. Movement of water between testa and cotyledon is restricted by coloured testa which adhered close to cotyledons while white testa adhered loosely allows rapid water movement (Powell *et al*., 1986; Dickson & Petzoldt, 1988). Cowpea seeds having pigmented seed coats imbibed rapidly when harvested at immature stage before the development of pigmentation. However, the rate of imbibition decreased markedly in cowpea when pigmentation of the seed coat developed during maturation (Legesse & Powell, 1996).

A significant positive correlation was found between WAR and 1000 seed weight (r=0.4937* in white and r=0.9718** in coloured seeds). The larger seeds absorbed more water than smaller ones. WAR showed negative correlation with seed coat ratio in both white (r=-0.1548) and coloured (r=-0.3057) seed group, but this relationships were not significant (Table 2).

Table 2. Correlations among WAR, EC, seed coat ratio, 1000 seed weight, laboratory germination and field emergence in both seed groups at 24 hours.

Cowpea genotypes		Seed coat ratio	1000 seed weight	Laboratory germination	Field emergence
White seeded[1]	EC	0.2172	-0.4475*	-0.0697	-0.7850**
	WAR	-0.1548	0.4937*	0.4726*	0.5842*
Coloured seeded[2]	EC	0.3750	-0.4410	-0.6653	-0.3261
	WAR	-0.3057	0.9718**	0.1008	-0.4663
All genotypes[3]	EC	0.1782	-0.4477*	-0.4512*	-0.5497**
	WAR	-0.3638	0.7709**	0.2969	0.1944

1)df=16-2=14, $r_{0.5}$=0.397, $r_{0.1}$=0.623 ; 2)df=5-2=3, $r_{0.5}$=0.878, $r_{0.1}$=0.959 ; 3 df=21-2=19, $r_{0.5}$=0.433, $r_{0.1}$=0.549

Electrical conductivity (EC): Differences in EC values of genotypes at the end of the 24 hours were highly significant (P<0.01). EC of white seeded cowpea genotypes increased linearly during 0-6 hours interval of imbibition. A very prominent increase occurred during 6-12 hours (Fig. 4 and 6).

Very low increases were observed in EC values of coloured seeds up to 6 hours. In these genotypes, the less permeable testa or high seed coat ratio may be the cause of slow water uptake. EC values of steep water for coloured seeds increased significantly during 6-24 hours (Fig. 5). Presently the seed coat permeability increased depending on time. However, structural deterioration of cell membrane may have occurred after 6 hours of imbibition.

EC of steep water showed similar trend to water uptake of seeds for both group (Fig. 6). This relationship was supported by changes of correlation coefficients depending on the time. Positive and significant correlations were found between EC (r=0.5352*) and WAR (0.6138*) in white seeded genotypes during the first 2 hour period of imbibition. However, significant positive relationship was found between EC (r=0.9460*) and WAR (0.9844**), of coloured seeded genotypes at 4-6 hours interval (Fig. 7). These periods were the peak times for the water absorption in both seed groups (Fig. 6).

Correlation between EC and WAR changed from positive to negative between 6-12 hours in white genotypes and during 12-18 hours in coloured genotypes (Fig. 7). At the end of 24 hours, conductivity readings as average of all genotypes negatively and significantly correlated with WAR (r=-0.4640*) (Fig. 7). EC values of seed steep water ranged from 18.62 µS/cm.g (G26) to 53.03 µS/cm.g (G23) among genotypes at the end of 24 hours (Fig. 4 and 5). There was a significant negative correlation between EC and 1000 seed weight (r=-0.4477*) (Table 2).

Laboratory germination and field emergence: The electrical conductivity test results can be related to field emergence. Kumar *et al*., (1989) reported that EC of *Vigna radiata* (L.) Wilezek seed exudates significantly correlated with final stand at maturity and EC and accelerated aging tests were the most important tests for predicting field emergence. Natarajaratnam *et al*., (1987) found that EC of seed leachates was negatively correlated with high yield. Seed leachates from low-yielding genotypes contained most sugars and free amino acids. It is suggested that suitable selection criteria for increasing yield would be EC and a vigour index based on shoot:root ratio and total dry matter 8 days after germination. In addition, Farghaly (1991) determined that seedling dry weight negatively correlated with EC and positively correlated with germination performance index, while germination performance index and EC was negatively correlated.

In the present study, laboratory germination percentages ranged from 87.33 to 100% (P<0.01). The lowest germination percentage was found in G23 cowpea genotype (87.33%). Germination percentage of the other genotypes were 93.67% and above (Fig. 8).

Field emergence started nearly at the end of the first week. FE was completed approximately after 17th day in all genotypes (Fig. 9). Very significant decreases occurred in FE when compared with LG in all genotypes. The lowest and highest field emergence percentage were 56.33% (G20) and 87.33% (GK), respectively (Fig. 8 and 9).

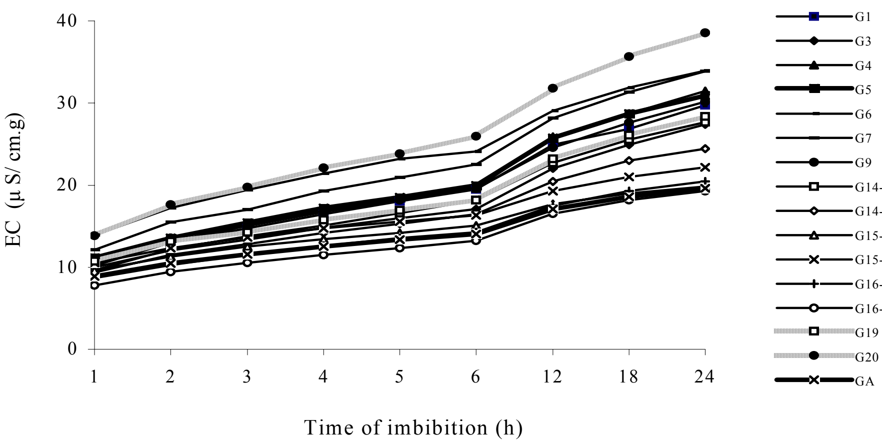

Fig. 4. Changes in electrical conductivity of steep water during the course of imbibition time of cowpea genotypes having white seed coats.

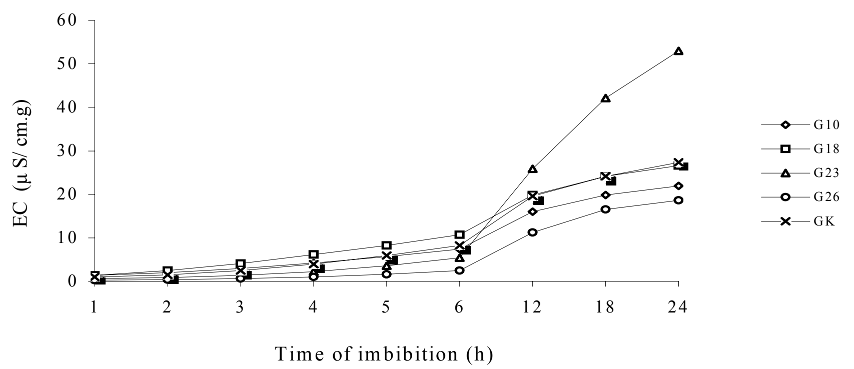

Fig. 5. Changes in electrical conductivity of steep water during the course of imbibition time of cowpea genotypes having coloured seed coat

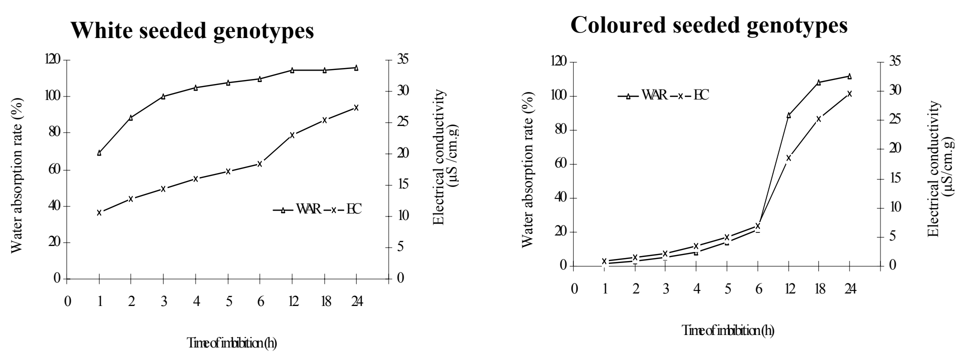

Fig. 6. Changes in WAR and EC of steep water during the course of imbibition time of cowpea genotypes having white and coloured seed coat

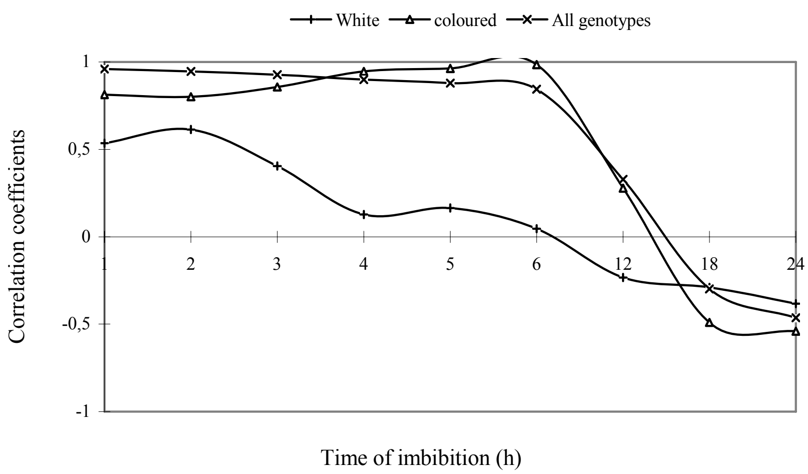

Fig. 7. Changing of correlation coefficients belonging to relationships between water absorption rate of cowpea seeds and electrical conductivity of steep water during the course of imbibition time.

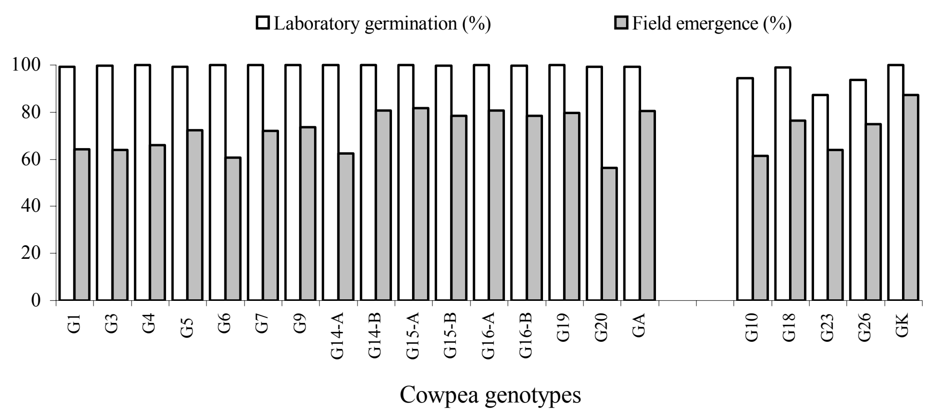

Fig. 8. Laboratory germination and field emergence percentages of cowpea genotypes.

Water uptake highly significantly correlated with LG (r=0.4726*) and FE (r=0.5842*) in white seeded genotypes. Looking at the average of all genotypes, the effect of WAR on LG (r=0.2969) and FE (r=0.1944) at the end of the soaking period was not significant. White seeded genotypes having high EC values had low performance for field emergence. There was a negative and highly significant correlation (r=-0.7850**) between EC and FE in white seeded genotypes, but this relationship was not significant in coloured genotypes. In an average of all genotypes, EC showed a negative and significant correlation with LG (r=-0.4512*) and FE (r=-0.5497**) at the end of the 24 hours (Table 2). This is in agreement with the reports of Kumar *et al*., (1989) and Farghaly (1991).

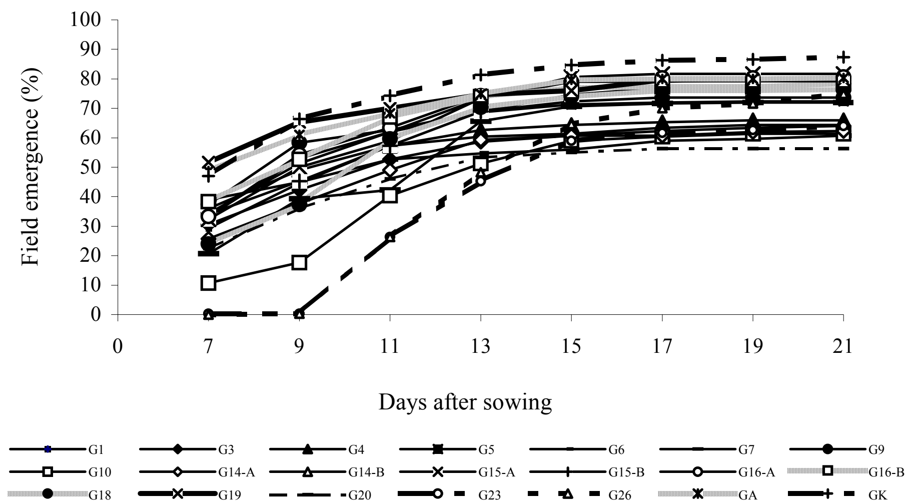

Fig. 9. Field emergence percentages of cowpea genotypes from 7 days to 21 days after sowing

The results of the present study showed that EC is an important test to predict field emergence potential of white seeded cowpea genotypes in the field conditions. Poor field emergence is one of the most important problems for cowpea in adverse soil conditions. High soil moisture and low soil temperature may cause a decrease in field emergence. Generally, rapid water uptake by white seeds results in increasing amounts of solute leakage from seeds to soil. For this reason, sowing of white seeds must be performed in more favourable soil moisture and temperature conditions than coloured seeds.

Acknowledgements

The authors wish to thank Prof. Dr. Ôzkan Sivritepe, Faculty of Agriculture, University of Uludag, Bursa-Turkey, for his valuable suggestions and critical reading of the manuscript.

References

Abdullah, W.D., A.A. Powell and S. Matthews. 1991. Association of differences in seed vigour in long bean with testa colour and imbibition damage. *Journal of Agric. Sci*., 116: 259-264.

Agrawal, P.K. 1986. Seed vigour. In: *Seed Production Technology*, (Eds.): J.P. Srivastava and L.T. Simarski, p: 190-198., ICARDA, Aleppo, Syria.

Anonymous. 1985a. International rules for seed testing. Rules 1985, *Seed Sci. and Technology*, 13: 299-355.

Anonymous. 1985b. International rules for seed testing. Rules 1985, *Seed Sci. and Technology*, 13: 356-513.

Burg, W.J. 1986. Aspects of seed quality control. In: *Seed Production Technology*, (Eds.): J.P. Srivastava and L.T. Simarski, p: 82-887., ICARDA, Aleppo, Syria.

Demir, İ. 1996. The effects of testa colour temperature and seed moisture content on the occurrence of imbibition damage in beans. *Tr. J. of Agriculture and Forestry*, 20: 295-298.

Dickson, M.H. and R. Petzoldt. 1988. Deleterious effect of white seed due to p gene in beans. *J. Am. Soc. Hort. Sci*., 113: 111-114.

Farghaly, M.A. 1991. Relationship of electrical conductivity of seed leachate to seed viability in cowpea. *Assiut Journal of Agricultural Sciences*, 22(3): 139-149 (CAB Abstracts 1993-7/95).

Gastel, A.J.G. 1986. Seed program components. In: *Seed Production Technology*, (Eds.): J.P. Srivastava and L.T. Simarski, p: 15, ICARDA, Aleppo, Syria.

Hampton, J.G and P. Coolbear. 1990. Potential versus actual seed performance- can vigour testing provide an answer?. *Seed Science and Technology*, 18: 215-228.

Kumar, R., C.S. Tyagi and C. Ram. 1989. Association among laboratory vigour tests and agronomic parameters in different genotypes of green gram (*Vigna radiata* (L.) Wilczek). *Seeds and Farms*, 15 (9-10): 22-27 (Seed-Abstracts 1990 013-00997).

Legesse, N. and A.A. Powell. 1992. Comparisons of water uptake and imbibition damage in eleven cowpea cultivars. *Seed Science and Technology*, 20(1): 173-180.

Legesse, N. and A.A. Powell. 1996. Relationships between the development of seed coat pigmentation, seed coat adherence to the cotyledons and the rate of imbibition during maturation of grain legumes. *Seed Science and Technology*, 24(1): 23-32.

Matthews, S. and W.T. Bradnock. 1967. The detection of seed samples of wrinkled-seeded peas (*Pisum sativum* L.) of potentially low planting value. *Proceedings of the International Seed Testing Association*, 32: 553-563.

Natarajaratnam, N., T.V. Rao and G. Pathmanabhan. 1987. Seed and seedling physiology of cowpea genotypes. *Madras Agricultural Journal*, 1985, 72(1): 31-34 (Seed Abstracts 1987 010-00488).

Pekşen, E., A. Pekşen, H. Bozoğlu and A. Gülümser. 2000. Değişik börülce (*Vigna unguiculata* (L.) Walp.) genotiplerinde bazı tohum özelliklerinin belirlenmesi. *O.M.Ü. Ziraat Fakültesi Dergisi*, 15(2): 65-72.

Powell, A.A., S. Matthews and M.De A. Oliveira. 1984. Seed quality in grain legumes. *Adv. Appl. Biol*., 10: 217-285.

Powell, A.A., M.De. A. Oliveira and S. Matthews. 1986. The role of imbibition damage in determining the vigour of white and coloured seed lots of dwarf french beans (*Phaseolus vulgaris* L.). *J. Exp. Bot*., 37(178): 716-722.

Sivritepe, H.Ö. and A.M. Dourado. 1995. The effect of seed moisture content and viability on the susceptibility of pea seeds to soaking injury. *Scientia Horticulturae*, 61: 185-191.

Srivastava, J.P. 1986. Status of seed production in the ICARDA Region. In: *Seed Production Technology*, (Eds.): J.P. Srivastava and L.T. Simarski, p: 1, ICARDA, Aleppo, Syria.

Yoshida, K., H. Sato and M. Sato. 1995. The extent of variation and its source for characteristics related to seed quality of adzuki beans. III. The water uptake of seeds and hardseededness. *Japanese Journal of Crop Science*, 64(1): 7-13.

(Received for publication 5 August 2002)