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Seed Quality and Vigor of Soybean as Influenced by Planting Date, Density and Cultivar under Temperate Environment

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Abstract: The objective of present study was to evaluate the relationship between different planting dates, densities and cultivars on soybean seed germination and vigor of seeds from plant growing in the field. Seeds of two soybean cultivars (Epps, [MG] V and Williams 82, [MG] III) were produced in the field from four planting dates and three planting densities in Peshawar, NWFP-Pakistan during 2000 and 2001. Brown (mature) pods were harvested, threshed and all shriveled and abnormal seeds were removed before determining standard germination and other vigor tests. Standard germination and other vigor tests decreased linearly (R^2 = 0.53) from early planting dates to delay planting dates. Similar trend of decrease was observed from low planting density to high planting density. The decrease in AA was curvilinear (R^2 = 0.78) and germination reached 15% in May planted crop. Seeds of Williams 82 was more sensitive to high temperature stress than seeds of Epps and seed vigor (AA) were much more sensitive to high temperature stress than was standard germination. Present findings support the results of experiments in controlled environments by demonstrating that high temperature during seed filling in the field, without seed infection with *P. longicolla* or physical injury, reduced soybean seed germination, vigor and all quality parameters of soybean seed under temperate environment.

Key words: Seed quality, vigor, germination, planting date, density, varieties, temperate, environment

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the important oil and protein crop of the world and is grown under a wide range of environmental conditions, where climatic factors such as temperature, photoperiod and moisture stress, exerts a detrimental effect on plant growth and metabolism. When grown as a summer crop, soybean usually experience gradually warming temperatures and lengthening days during the vegetative and reproductive period (Smithson *et al*., 1985). The timing of reproductive events in the crop and especially the duration of the blooming to physiological maturity period (growth stage R3 to R7) is modulated strongly by photoperiod and air temperature. High quality planting seed is a key component of all grain cropping systems and is needed to ensure adequate plant populations, with reasonable seeding rates, in a range of field conditions. Seed quality at planting represents the integrated effects of the environment during seed production and the conditions the seeds were exposed to during harvest, conditioning and storage. Unfavorable environmental conditions (temperature, rainfall, relative humidity) during seed growth and development

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in the field can reduce germination and vigor of soybean seed. High temperatures also reduced seed germination and vigor in growth chamber and phytotron experiments (Dornbos and Mullen, 1991; Zanakis *et al*., 1994a; Gibson and Mullen, 1996a). Temperatures of 33/28°C (day/night) (Keigly and Mullen, 1986), 35/30°C (Gibson and Mullen, 1996b), 38/33°C (Spears *et al*., 1997) and 38/27°C (TeKrony *et al*., 2000; Egli *et al*., 2005) during seed filling reduced germination of seed from several cultivars. Seeds produced by soybean plants exposed to excessively high temperatures during seed filling are shriveled or abnormal and the quality of these seeds is often much lower than seeds with no visible imperfection. Some reports suggest that drought stress during seed development also reduces seed quality (Smicklas *et al*., 1992; Heatherly, 1993), but Vieira *et al*. (1991, 1992) found no effect on germination or vigor in field and greenhouse experiments when the stress did not produce shriveled and abnormal seeds. Dry conditions at harvest may increase physical injury and reduce quality if seeds are handled at low moisture levels (TeKrony *et al*., 1984). Temperatures that reduced seed quality in controlled environments (32 to 38°C) could occur during seed filling in the field in many soybean production areas. It is difficult, however, to extrapolate the results of growth chamber and phytotron experiments to the field. Temperatures in the field vary diurnally and usually decrease during seed filling in temperate environments. Seed quality at harvest is primarily a function of disease, temperature and moisture conditions. We are not aware of reports describing effects of other aspects of the plant's environment (soil conditions, nutrient availability) on seed quality, so variation in seed quality in the absence of disease can be related to air temperature during seed development and maturation. Consequently, present objective was to evaluate the relationship between different planting dates, densities and cultivar on soybean seed germination and vigor of seeds from plants growing in the field under the temperate environment of NWFP-Pakistan.

MATERIALS AND METHODS

Site, Design and Sowing

The trials were conducted at Malakandher Farm, of the NWFP Agricultural University Peshawar (34° N latitude, 71.3° longitude) on a silty clay loam with a clay type montmorillonite, low in nitrogen (0.03-0.04%), low in organic matter (0.8-0.9%) and alkaline in reaction with a pH of 8.0-8.2 during 2000 and 2001. A basal dose of 36 kg N and 92 Kg P_2O_5 in the form of diammonium phosphate (DAP) fertilizer was applied at sowing. Indeterminate variety Williams 82 (MG-III) and determinate variety Epps (MG-V) were planted on May 2, June 2, July 2 and August 2. The experiment was laid out in randomized complete block design with split plot arrangement having four replications. Four planting dates were allotted to main plots, where as plant density and varieties were allotted to sub plots. A sub plot size of 4×5 m, having 8 rows five meters long was used. Sowing was done in hills and row to row distance of 50 cm and hill to hill distance of 10 cm were used. Normal cultural practices for raising a successful crop were followed uniformly for all the experimental units. Irrigation was applied at weekly intervals. Seeds from various planting dates, densities and varieties were evaluated for seed quality, immediately after harvest by various vigor tests i.e., by Standard germination test (SGT), accelerated aging (AA), electrical conductivity (EC), seedling axis dry weight (SADW) and expected field emergence test under stress conditions were carried out. For SGT, two 50 seeds samples from each field plot were planted in rolled paper towels and placed in a germinator at 25°C for 7 days (AOSA, 2002). The AA test was conducted as described by Hampton and TeKrony (1995). Two lots of 50 seeds from each treatment replicated four times were kept for 72 h at 42°C. Germination was determined after aging by planting 50 seed sample in rolled moist towels at 20°C as described by ISTA (1995) and final count was made at 7 days. For the conductivity test two replicates of 50 weighed seeds incubated for 24 h in 250 mL flask containing 200 mL of deionized water at 20±2°C. The electrical conductivity was measured with conductivity meter and expressed as µS cm^{-1} g^{-1} (ISTA, 1995). For the tetrazolium test, two replicates of 50 seeds from each treatment were stained with 10 g kg^{-1} solution of tetrazolium chloride according to ISTA method (ISTA, 1999). Seeds were evaluated and classified as viable or nonviable. Seedling axis dry weight (SADW) was determined by obtaining normal seedling on day 5th after germination in the dark. Cotyledons were detached before drying and were placed in paper bag, dried at 70°C for 48 h and weighed (AOSA, 2002). Field emergence test was carried out each year under stress environmental conditions with four replications of 100 seeds of each variety were planted by hand in 4 m rows at 3.5 cm depth. The plots were furrow irrigated to maintain relatively uniform soil moisture conditions. Daily counts were made as soon as the seedlings begin to emerge and continued until emergence was completed. Seedlings were considered as emerged, when the cotyledons were free of the soil surface. Soil temperature and average maximum and minimum air temperature at soil surface and one meter above the soil were recorded daily. An emergence index (EI) was calculated using the following formula:

$$EI = \{TiNi/S\}$$

Where Ti is the number of days after sowing, Ni is the number of seeds germinated on day I and S is the total number of seeds planted (Scott *et al*., 1984). Final emergence was calculated as a percentage of the number of seeds planted. All tests were performed by using a completely randomized design. Analysis of variance was performed and significant differences among treatments were determined by the LSD test at 0.05 level of probability.

RESULTS AND DISCUSSION

Standard Germination Test

Late planted crop of soybean gave maximum germination (81.8%), while early planted crop produced seeds with lowest germination percentage (Table 1). Maximum germination percentage of seeds from late planted crop indicates more viability and vigor than seeds from earlier planting. The higher germination percentage of seeds from delayed planted crops may be due to more protein content

Table 1: Standard germination test of soybean varieties as affected by date of sowing and plant density in 2000 and 2001

		Plants ha^{-1}			
		200	400	600	
Date of sowing	Variety	Two years average			Mean
D×V×P					
May 2	Epps	71.4	71.0	70.3	70.9d
	Williams 82	74.6	71.1	70.5	72.1d
June 2	Epps	72.9	73.8	71.0	72.5d
	Williams 82	76.5	76.8	69.8	74.3cd
July 2	Epps	81.5	84.4	76.9	80.9ab
	Williams 82	72.9	82.8	76.9	77.5bc
August 2	Epps	88.3	84.1	82.4	84.9a
	Williams 82	81.1	76.1	79.0	78.8bc
D×P					
May 2		73.0efg	71.1fg	70.4g	71.5c
June 2		74.7def	75.3de	70.4g	73.4c
July 2		77.2cd	83.6ab	76.9cde	79.2b
August 2		84.7a	80.1bc	80.7abc	81.8a
P×V					
	Epps	78.5	78.3	75.1	77.3
	Williams 82	76.3	76.7	74.0	75.7
Mean		77.4a	77.5a	74.6b	

*Means of the same category followed by different letter(s) are significantly different at 0.05% level of probability using LSD test in the seed and larger size of the embryo. These results are in line with those of Gibson and Mullen (1996a) and (Tekrony *et al*., 1984), who stated that late maturing varieties produced seed of high quality and seed vigor increased as harvest maturity was delayed. Delaying the planting date of early and mid season varieties improved seed germination and vigor and reduced the levels of seed infection by *Phomopsis* sp. Plant density significantly affected the germination percentage (Table 1) . Seed harvested from 20 and 40 plants m^{-2} gave higher germination than 60 plants per m^{-2}. Higher germination from lower plant densities may be attributed to less competition among plants for plant food material, more leaf area leading to greater total photosynthesis and more assimilates, which indirectly resulted in large and vigorous seed. The lower germination of seeds from thicker stand could be due to less light penetration in the canopy and more favorable conditions for seed infection during pre and post physiological maturity periods. No significant difference was observed between germination of the two varieties. The dates of sowing×varieties interaction reveal that seeds of both varieties produced at early May and early June planted plots did not significantly differ in germination percentage, though a linear increase in germination percentage was noted as sowing was delayed from May to June and Williams 82 gave higher germination percentage than Epps, while in late planted plots of July and August, the seed produced by Epps had significantly higher germination than seeds produced by Williams 82. The interaction between Dates of planting×plant population (Table 1) show that germination increased as sowing was delayed from May to August in the lowest and medium plant densities. Whereas, in the highest population, the germination of seed increase with delay in planting but the seed produced from the last planting date had lower germination than the seed produced by the penultimate date of sowing.

Accelerated Aging Test

Planting date had significantly affected the AA test (Table 2). Late planted crop gave higher germination (45.65%) than early planted crop (36.17%). The higher germination percentage of seeds from delayed planted crops may be due the greater variation in resistance to field weathering and tolerance to high temperature during germination which exists among varieties with different seed coat

Table 2: Accelerated aging test (percent germination) of soybean as affected by date of sowing and plant density in 2000 and 2001

Date of sowing	Variety	Plants ha^{-1} 200	400	600	Mean
		Two years average			
D×V×P					
May 2	Epps	37.88	35.88	31.25	35.00c
	Williams 82	39.75	39.00	33.25	37.33bc
June 2	Epps	38.00	34.38	34.25	35.54c
	Williams 82	44.25	37.00	33.88	38.38bc
July 2	Epps	46.13	39.88	37.13	41.04bc
	Williams 82	41.88	37.13	34.13	37.71bc
August 2	Epps	54.88	49.88	44.63	49.79a
	Williams 82	46.13	42.88	35.50	41.50b
D×P					
May 2		38.81	37.44	32.25	36.17b
June 2		41.13	35.69	34.06	36.96b
July 2		44.00	38.50	35.63	39.38b
August 2		50.50	46.38	40.06	45.65a
P×V					
	Epps	44.22	40.00	36.81	40.34
	Williams 82	43.00	39.00	34.19	38.73
Mean		43.61a	39.50ab	35.50b	

*Means of the same category followed by different letter(s) are significantly different at 0.05% level of probability using LSD test structures, seed sizes, seed weights and seed coat color (Tekrony *et al*., 1984; Miles *et al*., 1988), who stated that late maturing varieties produce seed of high quality and vigor. Later plantings of early and mid season varieties improved seed germination and vigor and reduced the levels of seed infection by *Phomopsis* sp. Low plant density of 20 and 40 plants m^{-2} gave higher germination percentage than 60 plants per m^{-2} in accelerated aging test. Higher germination percentage from lower plant densities may be attributed to vigorous seed, which showed resistance to stress conditions as compared to higher planting density. The lower germination of seeds from thicker stand could be due to more favorable conditions for seed infection during seed filling duration. Averaged over the planting dates and populations, no significant difference was observed between germination of the two varieties. The dates of sowing x varieties interaction reveals that seeds of both varieties produced during early May and June planted plots did not significantly differ in germination percentage after accelerated aging. Williams 82 gave higher germination percentage than Epps, while in late planted plots of July and August, the seed produced by Epps had significantly higher germination than Williams 82.

Tetrazolium Vigor Index

The statistical analysis of the data showed that planting dates significantly affected TZ vigor index (Table 3). Early planted crop of soybean had the lowest TZ vigor index of 35.9 and the vigor index increased as sowing was delayed till last week of August (45.8). High vigor index from late planted crop may be due to comparatively lower temperature during seed development. This is further confirmation of the result of electrical conductivity of the seed leachate where late planting has minimum electrical conductivity. No significant difference was observed between varieties and plant densities with respect to TZ vigor index. Dates of planting x plant densities interaction reveals that in May planted plots TZ vigor index decreased as planting density increased. In early July planted plots TZ vigor index increased with increase in population density. In early August planted plots, plant density did not significantly affect TZ vigor index. The dates of planting×varieties interaction means show that the differences between TZ vigor index of Epps and Williams 82 planted in early May and July were not significant, while the difference between the TZ vigor index of the seeds of two varieties from early June and early July plantings were significant but had different ranking. Seeds of Williams 82 planted in early June had better TZ vigor index than Epps, but in August planted plots, seeds of Epps had better TZ vigor index than Williams 82.

Table 3: Tetrazolium vigor index of soybean varieties as affected by date of sowing and plant density in 2000 and 2001

		Plants ha^{-1}			
		200	400	600	
Date of sowing	Variety	Two years average			Mean
D×V×P					
May 2	Epps	40.9	34.6	33.2	36.2d
	Williams 82	36.8	37.0	32.8	35.5d
June 2	Epps	37.5	34.4	37.6	36.5d
	Williams 82	38.7	39.5	40.1	39.4c
July 2	Epps	34.7	41.3	45.0	40.3c
	Williams 82	38.6	40.7	46.3	41.9bc
August 2	Epps	48.8	49.2	46.1	48.0a
	Williams 82	44.3	43.9	42.3	43.5b
D×P					
May 2		38.9b	35.8de	33.0e	35.9d
June 2		38.1cd	36.9d	38.8cd	38.0c
July 2		36.6de	41.0bc	45.7a	41.1b
August 2		46.5a	46.5a	44.2ab	45.8a
P×V					
	Epps	40.5	39.9	40.5	40.3
	Williams 82	39.6	40.2	40.4	40.1
Mean		40.0	40.1	40.4	

Electrical Conductivity

Maximum conductivity of 33.1 Micro-siemens $cm^{-1}g^{-1}$ of seed leachate was recorded in early planted crop of soybean (Table 4). Delay planted crop produced seeds with the minimum electrical conductivity. Maximum electrical conductivity of seed leachate from early planted crop may be due to high day temperature during seed filling duration and at physiological maturity as compared to late planted crop. No significant difference were observed between the electrical conductivity of the leachate of seeds produced by the three plant densities, however lowest plant density of 20 plants m^{-2} produced seeds with greater electrical conductivity of seed leachate of 23.6 Micro-siemens $cm^{-1}g^{-1}$ than higher plant density of 60 plants m^{-2}. Seeds developed in the plots with low plant density were exposed to comparatively more direct sunlight, high day temperatures and greater diurnal temperature amplitude due to lower canopy density than higher plant densities. These findings are in agreement with those of Bhering *et al*. (1991) and Zanakis *et al*. (1994b) who reported that seed quality was generally better with later sowing dates than earlier sowing dates because the maturation of early planted crop coincided with high rainfall and temperature. No significant difference was observed between varieties with respect to electrical conductivity, however variety Epps indicate more conductivity of seed leachate than Williams 82.

Seedling Dry Weight

Maximum seedlings dry weight of 4.0 mg per 50 seeds were obtained from early planted crop and a steady decrease in seedlings dry weight was noted as sowing was delayed (Table 5). Maximum seedlings dry weight from early planted crop indicates that seed produced from early planting dates were heavier in seed size and due to long growth period, which ultimately resulted in more seedlings dry weight than late planted crop (Caulfield and Bunce,1991). Seedlings dry weight was significantly different among varieties as seeds of Williams 82 produced significantly more seedlings dry weight than Epps. Plant density of 20 plants m^{-2} produced more seedlings dry weight followed by seedlings dry weight of 40 plants m^{-2}. Differences between seedling dry weight of 40 and 60 plants m^{-2} was not significant. Maximum seedlings dry weight from lowest plant density may be due to less competition

Table 4: Electrical conductivity (micro-siemens $cm^{-1}g^{-1}$) of soybean varieties as affected by date of sowing and plant density in 2000 and 2001

		Plants ha^{-1}			
		200	400	600	
Date of sowing	Variety	Two years average			Mean
D×V×P					
May 2	Epps	37.4	30.8	29.6	32.6
	Williams 82	39.0	32.3	29.6	33.6
June 2	Epps	36.7	32.4	35.5	34.9
	Williams 82	24.2	25.8	21.2	23.7
July 2	Epps	15.4	12.9	15.3	14.6
	Williams 82	15.1	12.8	11.5	13.1
August 2	Epps	8.7	11.2	12.4	10.8
	Williams 82	12.6	11.5	14.7	13.0
D×P					
May 2		38.2	31.5	29.6	33.1a
June 2		30.5	29.1	28.3	29.3b
July 2		15.3	12.9	13.4	13.9c
August 2		10.7	11.3	13.6	11.9d
P×V					
	Epps	24.5	21.8	23.2	23.2
	Williams 82	22.7	20.6	19.3	20.9
Mean		23.6	21.2	21.2	

Means of the same category followed by different letter(s) are significantly different at 0.05% level of probability using LSD test among plants for the availability of plant food material from the soil and maximum leaf area plant^{-1} with greater photosynthesis, which ultimately resulted in heavier seedlings as compared to thick densities of soybean.

Expected Field Emergence

Minimum percent field emergence of 57.7 was given by seeds obtained from early planting (Table 6). A steady increase in field emergence percentage occurred with delay in planting and the late planting date produced seeds with maximum field emergence percentage of 70.3%. The maximum

Table 5: Seedlings dry weight (mg/50 seeds) of soybean varieties as affected by date of sowing and plant density in 2000 and 2001

		Plants ha^{-1}			
		200	400	600	
Date of sowing	Variety	Two years average			Mean
D×V×P					
May 2	Epps	3.9	3.6	3.6	3.7bc
	Williams 82	4.4	4.3	4.1	4.3a
June 2	Epps	3.7	3.4	3.2	3.4cd
	Williams 82	4.0	4.0	3.9	3.9ab
July 2	Epps	3.6	3.4	3.2	3.4cd
	Williams 82	3.6	3.5	3.6	3.6bc
August 2	Epps	3.4	3.0	2.7	3.0d
	Williams 82	3.9	3.0	3.0	3.3cd
D×P					
May 2		4.2	4.0	3.8	4.0a
June 2		3.8	3.7	3.5	3.7b
July 2		3.6	3.5	3.4	3.5b
August 2		3.6	3.0	2.8	3.2c
P×V					
	Epps	3.6	3.4	3.2	3.4b
	Williams 82	4.0	3.7	3.6	3.8a
Mean		3.8a	3.5b	3.4b	

Means of the same category followed by different letter(s) are significantly different at 0.05% level of probability using LSD test

Table 6: Field emergence (%) of soybean varieties as affected by date of sowing and plant density in 2000 and 2001

		Plants ha^{-1}			
		200	400	600	
Date of sowing	Variety	Two years average			Mean
D×V×P					
May 2	Epps	59.4	52.4	56.3	56.0f
	Williams 82	60.5	58.3	59.3	59.3ef
June 2	Epps	61.1	62.3	58.3	60.5de
	Williams 82	63.6	62.6	57.3	61.2de
July 2	Epps	64.4	64.8	63.4	64.2cd
	Williams 82	63.6	77.8	62.1	67.8b
August 2	Epps	78.6	72.9	69.1	73.5a
	Williams 82	72.5	64.4	64.1	67.0bc
D×P					
May 2		59.9e-g	55.3g	57.8fg	57.7d
June 2		62.4d-f	62.4d-f	57.8fg	60.9c
July 2		64.0c-e	71.3ab	62.8d-f	66.0b
August 2		75.6a	68.6bc	66.6b-d	70.3a
P×V					
	Epps	65.9a	63.1ab	61.8b	63.6
	Williams 82	65.1a	65.8a	60.7b	63.8
Mean		65.5	64.4	61.2	

Means of the same category followed by different letter(s) are significantly different at 0.05% level of probability using LSD test percentage of field emergence from late planting may be due to optimum temperature during seed development and maturation in October and November and due to more protein content, which ultimately helped during germination and emergence of the seedlings. These results are in agreement with those of Steiner (1990) who stated that high vigor seedlots have significantly higher emergence than low vigor seedlots. Plant densities showed no significant effect on field emergence of the soybean seeds; however plant density of 20 plants m^{-2} gave higher field emergence than higher plant densities. Interaction between dates of sowing×varieties indicate that at early planting dates seeds of Williams 82 had better emergence than Epps, however at late planting date seeds of Epps showed better emergence than Williams 82. Dates of planting×plant population interaction means show that in the lowest and highest plant densities, a linear increase in field emergence was noted when seeds from early to late planting dates were sown in field. However, in medium plant density the response of field emergence from last sowing date was not linear as there was a significant decrease in field emergence of seeds from the last planting date.

CONCLUSIONS

It is concluded from this study that for quality seed production (viability and vigor) soybean should be planted in the first week of August at the rate of 2,00,000 plants ha^{-1} in areas, where temperature is milder as that experienced by crops planted late under the climatic conditions of Peshawar valley.

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