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Response of germination to seed size and color of pigeonpea [*Cajanus cajan* (L.) Millsp.]

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ABSTRACT

The effect of large and small seed size and color of pigeonpea on germination was studied on six hybrids and twelve inbred lines at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. Seeds of each genotype were segregated into large and small seed classes according to their 100-seed weight (g). Experiment revealed that seed size had an effect on rate of germination up to seven days after sowing. In addition, seed color significantly affected germination of pigeonpea in day second and day third. Although there was a mixed response among individual genotypes, large seeds germinated significantly earlier in ICEAP 000040-1, ICP 11811, ICP 13395, and ICP 7035. However, germination percentage of small seeds among the other genotypes was not significantly affected during day eight, indicating uniform performance with the large seeds. The results of this study showed that small seeds can likewise be used as planting material, whereby farmers can gain more seeds per unit weight as compared to large seeds while maintaining the same germination performance. However, the relationship between seed size and germination is complex and differs from species to species and even differs between each cultivar within a species.

Key words : *Cajanus cajan, Genotypes, Germination, Hybrid, Pigeonpea, Seed size, Varieties.*

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is grown by smallholder farmers in over 4.6 million hectares in the tropics and subtropics of India, Myanmar, Kenya, Malawi, Uganda, and a few countries of Central America and the Caribbean. Because it is rich in vitamins and minerals and has high protein content (22-24%), pigeonpea plays a significant role in securing food and nutritional security of the Indian population. Besides food, pigeonpea can also be used for animal feed, forage, fuel wood, living fences, and as a culture for the lac-producing insect. It provides several benefits to the soil, fixing nitrogen, adding organic matter by leaf fall, and improving soil structure by breaking through plough pans (Mula and Saxena 2010).

In general, farmers tend to sow large seeds with hesitation towards small seeds, as they doubt behavioral germination and uniform plant stand establishment. Size of seeds is an important characteristic which is affected by variety, environment and management practices (Robinson, 1978). However, the effectiveness of using large or small seeds as a source of planting material has mixed reactions from previous research. In principle, seed size has effects on many characters both in the field and laboratory tests. Percent of seed emergence and speed of seedling emergence are the characteristics that could be observed in the field and could be used as an indicator of seed vigor. Although genetic background of seeds in individual lines or cultivars is similar, seed sizes may affect other agronomic characters. These characters usually differ under field stresses such as low temperature, wet soil, or crusted soil. Studies have shown that under such stresses, small seeds of soybean and common bean perform better than large (Hoy and Gamble, 1987; Sexton *et al*., 1994). In addition, Suh *et al*. (1974) explained that the difference in seed size and seed weight on the resulting crop was not affected by genetic and quality difference. These results imply that seed weight would have little influence on yield of the resulting crop.

White and Singh *et al*. (1992) found that small-seeded genotypes were physiologically most efficient over large-seeded genotypes in common bean at warmer areas and higher latitudes. But, Singh *et al*. (1972) reported that large seeds of soybean had greater supply of stored energy to support early seedling growth and subsequently affected plant growth and development. However, seed size has been considered to be a significant factor only during the early stage of plant growth. Nevertheless, Amin (1999) reported that 50%

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of large-seeded mungbean matured earlier than that of small-seeded type. Large seed has an advantage of having higher stored energy supply, but not all reports demonstrated the effects of seed size on yield. Although the largest seed sizes have the largest cotyledon area, the higher photosynthetic rate from smaller seed size could compensate and support seedling growth (Burris *et al*., 1973).

According to Tully *et al*. (1981), black-pigmented seed coats imbibed water more slowly than non-pigmented seeds. Because imbibition takes longer, germination of dark-colored seeds is delayed behind that of light-colored seeds.

For past five decades, there was no reported research on the effect of seed size on germination until Bharathi and Saxena (2012) reported that pigeonpea varieties with larger seed size had increased germination percentage and seedling vigor. The purpose of this study was to trace the seed laboratory germination test on the effect of seed size within the same pigeonpea varieties and hybrids on the speed of emergence and the efficiency of germination.

MATERIALS AND METHODS

The laboratory study was conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India on June 8 to June 16, 2013. Six hybrids and twelve inbred lines of pigeonpea were evaluated to determine the effect of seed size and seed color on germination character. For each genotype, seed was selected from bulk source (ICRISAT) and categorized as small and large seeds by recording 100 seed weight (g) (**Table 1**).

The germination test was conducted using the petri dish method. Germination paper (Whatman Grade 181) was placed in 9-cm petri dishes and moistened with distilled water until saturated before sowing. Each genotype with smaller and larger seed was sown separately with three replications. Fifty seeds of each genotype treated with tetra methyl thiuram disulphide at 2.5 g/kg against fungal disease were evenly sown on the surface of the germination paper in petri dishes. The tops of the petri dishes were covered with germination paper and moistened with water to maintain the humidity. The petri dishes were kept at room temperature (25°C) under normal light. Germination paper was moistened with water at 24 hour intervals from time of sowing to keep adequate moisture levels. Germinated seeds were counted at 24 hour intervals starting from the second day of testing and continuing until the eight day. Seeds were considered germinated when both radicle and plumule had emerged to about 2 mm. Affected seeds caused mainly by fungal disease where discarded and counted as non-germinated seeds.

Data for germination percentage was analyzed using analysis of variance (ANOVA) technique considering factorial experiment of genotype and seed size in a completely randomized design (CRD). ANOVA was carried out using the glm (Generalized Linear Model) procedure of the SAS software version 9.3 for Windows (SAS Institute Inc. 2011, Cary, NC). Interaction effect of genotype x seed size was tested for significance and significance of main effects of genotype and seed size was interpreted based on crossover/non-crossover genotype x seed size interaction. Data was further analyzed by using ANOVA to study the effect of seed color on germination percentage on each day. Least Square Means of each color were estimated and compared using least significant differences with the use of t-grouping.

Table 1. List of genotypes and their characters

Parti-culars	Genotypes	Seed color	100 seed weight (g)		Type	Duration
			Small	Large		
Hybrids						
	ICPH 2671	Purple	10.0	12.2	NDT	Medium
	ICPH 2740	Brown	7.2	10.0	NDT	Medium
	ICPH 3762	Brown	7.8	11.9	NDT	Medium
	ICPH 4503	Cream	11.6	14.7	NDT	Medium
	ICPH 4182	Cream	8.7	11.4	NDT	Medium
	ICPH 4431	Cream	8.0	11.7	NDT	Medium
Inbred-lines	ICPL 161	Brown	5.4	8.5	NDT	Early
	ICPL 88039	Brown	8.0	9.6	NDT	Early
	ICP 8863	Brown	9.5	11.2	NDT	Medium
	ICPL 87119	Brown	10.1	12.0	NDT	Medium
	ICP 12057	Light brown with brown	11.3	13.2	NDT	Medium
	ICP 13395	Light brown	18.6	20.5	NDT	Medium
	ICP 13384	Light brown	18.3	20.4	NDT	Medium
	ICPL 87091	Cream	10.0	14.1	DT	Medium
	ICP 14304	Cream	10.4	12.6	NDT	Medium
	ICEAP 000040-1	Cream	18.9	20.4	NDT	Medium
	ICP 7035	Purple	18.5	23.0	NDT	Medium
	ICP 11811	Black	13.3	16.9	NDT	Medium

Note : NDT - Non-determinate; DT - Determinate

RESULTS AND DISCUSSION

Variation of seed size : A significant difference was evident between the 100-seed weights of large and small seeds within every pigeonpea genotype (**Table 1**). The weight difference between small and large seeds varies within genotype from as low as 1.5 g (ICEAP 000040-1) to as high as 4.5 g (ICP 7035).

Effect of seed size : The response of germination on seed size was significant except for day eight, where both large and small seeded pigeonpea performed uniformly (**Table 2**) which is in accordance to the findings of Emenky and Khalaf (2008) and Kaya and Day (2012). **Table 3** showed that large-seeded genotypes significantly outperformed small-seeded genotypes on day two by 13.7%. On day three, large seeded-genotypes still had a significantly higher germination, although only by 7.9%. The difference between large and small seeds continued to be significant, but decreased each day (4.3% on day four, 2.7% on day five, 1.7% on day six, and 1.3% on day

Table 2. Analysis of variance (ANOVA) for germination (%) across genotype and seed size

Source of variation	DF	Germination percentage						
		Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Genotype	17	0.62025**	0.37053**	0.16696**	0.16282**	0.19267**	0.18478**	0.19198**
Seed size	1	0.80457**	0.56282**	0.30859*	0.18448**	0.08936*	0.05894*	0.04119
Genotype x seed size	17	0.16812*	0.16784**	0.05504	0.03616**	0.03220**	0.03528**	0.02886**
Error	64	0.07999	0.06571	0.04968	0.01532	0.01296	0.01332	0.01244

Note : * Significant at P = 0.05, ** Significant at P = 0.01

Table 3. Mean performance of pigeonpea seed sizes for germination (%) irrespective of genotype

Size	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Large	72.1**	89.1**	94.6*	96.3**	96.7*	96.7*	96.8
Small	58.4	81.2	90.3	93.6	95.0	95.4	95.7

Note : * Significant at P = 0.05, ** Significant at P = 0.01

seven). By day eight, the difference was no longer significant and small seeds were performing just as well as large seeds.

Effect of genotype : **Table 2** revealed that the response of genotype on percent germination was significant during the test period. On day two, ICPL 161 had the highest germination at 95.4% while ICP 13384 had lowest germination at 8.7% (**Table 4**). On day three ICP 14304 performed best at 97.7% germination while ICEAP 000040-1 had the lowest germination with 55.5%. On day four and five, ICP 14304 had the highest germination with 98.7% and 98.9%, respectively while ICPH 4431 had the lowest germination with 74.1% and 75.1%, respectively. ICEAP 000040-1 performed best in the later stages of germination with 99.8%, 99.8%, and 99.9% for day six, seven, and eight, respectively while ICPH 4182 had the lowest germination with 76.6% in day six and 76.9% for days seven and eight.

Table 4. Mean performance of pigeonpea genotypes for germination (%) irrespective of seed size

Genotypes	Mean seed size (g)	Days						
		2	3	4	5	6	7	8
ICPH 2671	11.1	60.3	90.7	94.3	96.9	96.9	96.9	96.9
ICPH 2740	8.6	77.2	85.9	88.4	90.2	90.2	90.6	90.6
ICPH 3762	9.85	89.8	89.8	91.3	92.7	91.9	92.2	92.5
ICPH 4503	13.15	68.3	90.9	95.8	96.5	96.9	96.9	97.3
ICPH 4182	10.05	64.2	74.2	76.0	76.6	76.6	76.9	76.9
ICPH 4431	9.85	56.3	70.2	74.1	75.1	76.8	78.3	78.6
ICPL 161	6.95	95.4	96.1	96.1	96.6	96.6	96.6	96.6
ICPL 88039	8.8	87.7	92.3	94.4	95.3	95.8	97.6	97.9
ICP 8863	10.35	76.5	96.8	97.3	98.1	99.2	99.2	99.2
ICPL 87119	11.05	86.0	96.5	97.1	97.1	97.4	97.7	97.7
ICP 12057	12.25	75.7	94.9	97.2	97.7	98.3	98.3	98.3
ICP 13395	19.55	25.7	68.5	95.2	98.1	99.3	99.3	99.7
ICP 13384	19.35	8.7	60.0	91.4	96.7	97.0	97.0	97.0
ICPL 87091	12.05	65.2	93.3	98.3	98.9	98.9	98.9	99.1
ICP 14304	11.5	85.3	97.7	98.7	98.9	98.9	98.9	98.9
ICEAP 000040-1	19.65	20.6	55.5	86.1	96.7	99.8	99.8	99.9
ICP 7035	20.75	28.5	64.2	84.7	96.5	98.1	98.1	98.3
ICP 11811	15.1	48.0	70.8	87.6	89.9	90.4	90.4	91.4

Interactive effect of genotype and seed size : The interaction of genotype and seed size was significant in the early and late stages of germination except for day four, where the interaction of genotype and seed size did not have a significant effect (Table 2). Excluding day eight, the overall

Table 5. Mean performance of pigeonpea genotypes for germination (%) with varying seed size

Genotypes	Size	Days						
		2	3	4	5	6	7	8
ICPH 2671	Large	71.0	90.0	95.5	98.9	98.9	98.9	98.9
	Small	48.4	91.4	92.9	93.9	93.9	93.9	93.9
ICPH 2740	Large	59.5	80.7	84.9	88.1	88.1	88.9	88.9
	Small	90.3*	90.3	91.5	92.1	92.1	92.1	92.1
ICPH 3762	Large	93.8	92.4	93.4	93.4	94.0	94.0	94.0
	Small	84.8	86.9	88.9	92.1	89.5	90.3	90.8
ICPH 4503	Large	90.2**	92.7	97.3	97.7	97.7	97.7	98.3
	Small	37.2	89.0	94.0	95.0	96.0	96.0	96.0
ICPH 4182	Large	72.2	76.7	78.8	79.9	79.9	80.5	80.5
	Small	55.4	71.5	73.0	73.0	73.0	73.0	73.0
ICPH 4431	Large	66.7	78.0	80.1	80.1	81.6	81.6	81.6
	Small	44.9	61.4	67.5	69.6	71.5	74.8	75.4
ICPL 161	Large	94.4	95.0	95.0	96.2	96.2	96.2	96.2
	Small	96.3	97.1	97.1	97.1	97.1	97.1	97.1
ICPL 88039	Large	87.0	90.3	92.2	93.0	94.3	94.3	95.2
	Small	88.3	94.2	96.3	97.1	97.1	99.5*	99.5
ICP 8863	Large	89.9*	95.7	96.9	96.9	96.9	96.9	96.9
	Small	58.6	97.7	97.7	99.1	100.0*	100.0*	100.0
ICPL 87119	Large	91.0	97.9	97.9	97.9	97.9	97.9	97.9
	Small	80.1	94.8	96.2	96.2	96.7	97.4	97.4
ICP 12057	Large	80.0	94.7	98.0	98.0	98.0	98.0	98.0
	Small	71.0	95.0	96.2	97.4	98.7	98.7	98.7
ICP 13395	Large	49.0	97.1**	100.0**	100.0**	100.0*	100.0*	100.0
	Small	0.7	18.1	81.8	92.9	97.4	97.4	99.1
ICP 13384	Large	5.3	58.9	92.5	98.2	98.7	98.7	98.7
	Small	12.0	61.0	90.1	94.8	94.8	94.8	94.8
ICPL 87091	Large	46.8	88.6	96.9	97.4	97.4	97.4	98.0
	Small	80.3	96.8	99.3	99.7	99.7	99.7	99.7
ICP 14304	Large	79.5	96.4	98.7	98.7	98.7	98.7	98.7
	Small	90.2	98.7	98.7	99.1	99.1	99.1	99.1
ICEAP 000040-1	Large	37.8	82.3**	95.3*	99.5**	100.0	100.0	100.0
	Small	2.7	21.0	72.6	91.8	99.3	99.3	99.7
ICP 7035	Large	47.7	78.7	91.4	99.0*	99.7*	99.7*	99.7
	Small	8.0	46.9	76.4	92.4	95.0	95.0	95.6
ICP 11811	Large	76.1**	89.0**	95.5	96.3**	96.3**	96.3**	96.3
	Small	13.6	45.7	76.2	80.7	82.1	82.1	84.7
Overall	Large	72.1**	89.1**	94.6*	96.3**	96.7*	96.7*	96.8
	Small	58.4	81.2	90.3	93.6	95.0	95.4	95.7

Note : * Significant at P = 0.05, ** Significant at P = 0.01

mean germination percentage of genotypes across the test period was significantly different where large-seeded genotypes outperformed the small-seeded genotypes (**Table 5**) which is in conformity to the findings of Marshall (1986), Burris *et al*., (1973) and Emenky and Khalaf (2008) where large seeds have higher vigor due to its stored food reserves.

However, there are many differing results with regards to seed size effect on germination in the individual pigeonpea genotypes. For medium duration cultivars, large seeds of ICEAP 000040-1 germinate faster during day's three to five but were not significantly different with the small seeds during the rest of the test period (**Fig. 1**). For ICP 11811, the large seeds were dominant and outperformed the small seeds during the test period (**Fig. 2**). Large seeds of ICP 13395 performed better than the small seeds until day seven, except in day eight where the germination of both type of seeds was uniform (**Fig. 3**). In ICP 7035, large seeds showed higher germination percentage during day five to seven (**Fig. 4**), which is in conformity to the findings of Marshall (1986). However, the large seeds of ICP 8863 showed that the germination was higher during day two but was outdone by the small seeds during day 6 & 7 (**Fig. 5**).

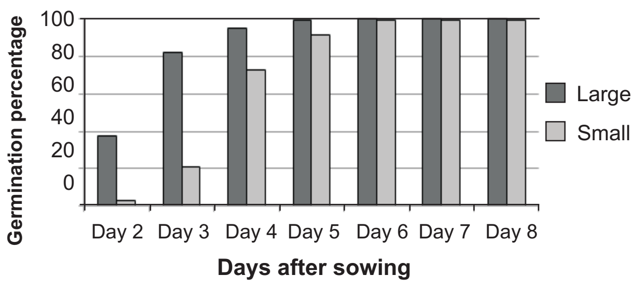

Fig. 1. Germination percentage for large and small seeds of ICEAP 000040-1

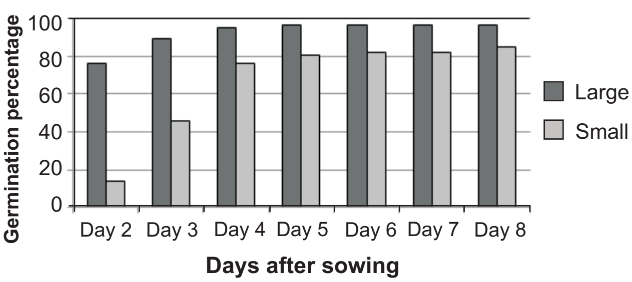

Fig. 2. Germination percentage for large and small seeds of ICP 11811

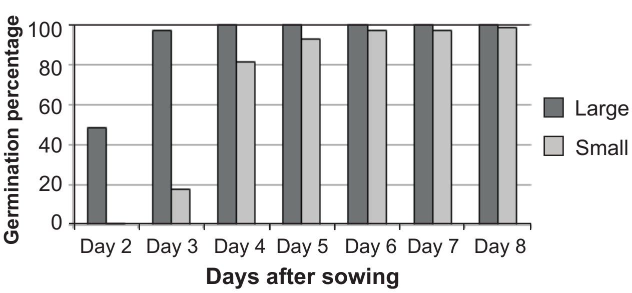

Fig. 3. Germination percentage for large and small seeds of ICP 13395

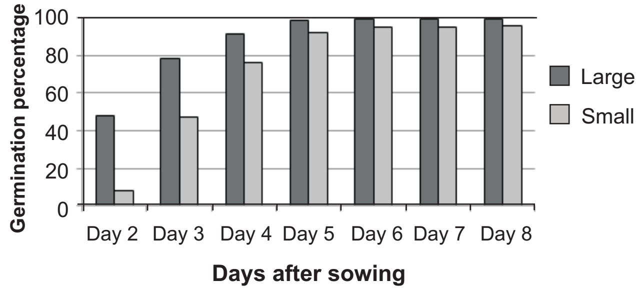

Fig. 4. Germination percentage for large and small seeds of ICP 7035

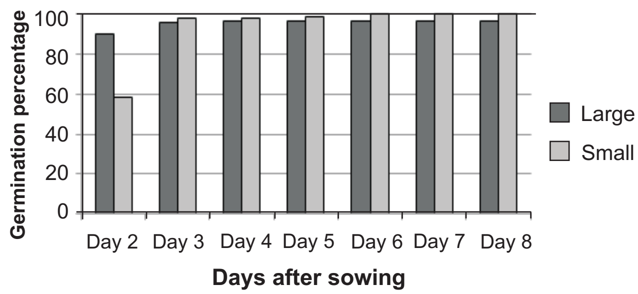

Fig. 5. Germination percentage for large and small seeds of ICP 8863

For early duration cultivars, small seeds of ICPL 88039 performed better (**Table 5**) which follows the findings of Edwards and Hartwig (1971).

Within hybrids, the performance of large and small seeds was not significantly different, except for the large seeds of ICPH 4503 during day two. For small seeds, ICPH 2740 showed a significant difference during day two (Table 5).

Effect of seed color : Irrespective of seed size and genotype, seed color showed a highly significant effect on the germination of pigeonpea in day two and three (**Table 6**). **Table 7** revealed that on both days (two and three), brown and light brown with brown seeds had the highest germination percentage, followed by cream, purple, black, and light brown (**Fig. 6**) which conforms to the findings of Tully *et al*. (1981).

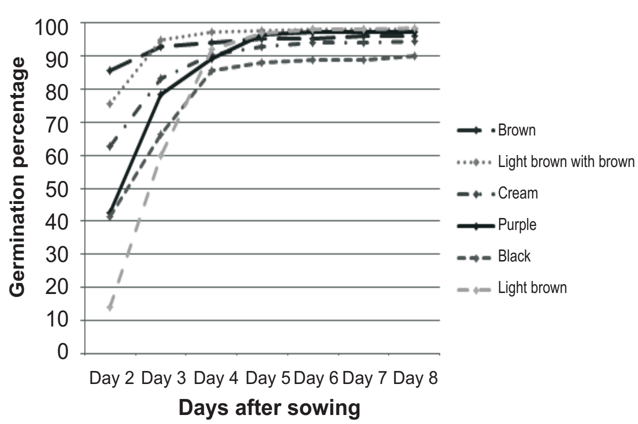

Fig. 6. Mean performance of color for germination percentage

Table 6. Analysis of variance (ANOVA) for germination (%) across seed colors of pigeonpea genotypes

Source of variation	DF	Germination percentage Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Color	5	1.64873**	0.71302**	0.09597	0.08657	0.09571	0.09272	0.08926
Error	94	0.12500	0.11891	0.07275	0.04340	0.04529	0.04448	0.04420

Table 7. Mean performance of seed color of pigeonpea genotypes on germination (%)

Color	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Brown	85.6 a	93.1 a	94.3 a	95.2 a	95.5 a	96.0 a	96.1 a
Light brown with brown	75.7 ab	94.9 ab	97.2 a	97.7 a	98.3 a	98.3 a	98.3 a
Cream	62.9 bc	83.1 bc	90.4 a	92.8 a	94.1 a	94.3 a	94.6 a
Purple	42.5 cd	78.3 cd	89.4 a	96.5 a	97.4 a	97.4 a	97.5 a
Black	41.5 ce	66.2 cd	85.6 a	88.3 a	89.0 a	89.0 a	90.2 a
Light brown	14.2 de	59.9 d	92.2 a	97.0 a	98.1 a	98.1 a	98.5 a

Note : Means followed by the same letter (*a-e*) in a column do not differ significantly at $P = 0.05$

CONCLUSION

The present study revealed that in pigeonpea generally large seeds germinate faster than small seeds, especially within ICEAP 000040-1, ICP 11811, ICP 13395, and ICP 7035. After eight days, the effect of seed size is insignificant, as small seeds germinate just as well as large seeds. In this regard, small seeds are more advantageous to farmers due to having more number of seeds per unit weight thus this will allow the farmers to reduce their input cost on seeds.

The relationship between seed size and germination is complex and differs from species to species. According to the present study, the relationship even differs between each cultivar within a species. Furthermore, the effects of seed size may be apparent only under competitive conditions (Black 1958). Conditions within the petri dish were not competitive, suggesting that results may turn out differently under competitive conditions. Further research may be conducted on the effect of seed size under field conditions such as drought stress or under high populations.

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