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Evaluation of somaclones derived from *in-vitro* culture induced somatic tissues in pigeonpea

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ABSTRACT

Fifteen somaclonal variants developed from adventitious shoot-derived callus from the cotyledonary explants of pigeonpea [*Cajanus cajan* (L.) Millsp.] var. 'ICPL 87' were assessed across two seasons for some agronomic and cooking quality characters. The mutant inbreds showed significant variation for days to maturity, plant height, seed size, seed colour and grain yield. Some of the somaclonal variants such as 'ICPL 99073', 'ICPL 99072' and 'ICPL 99070' were found promising and displayed significant positive changes for some important agronomic traits. Those include a change from small seed size and brown seed coat colour to more preferred large white seeds endowed with more seed yield. For grain yield, 'ICPL 99073' showed 25.3% yield advantage over the parent variety. The studies demonstrated the scope of genetic improvement in pigeonpea through deployment of somatic culture and exploitation of somaclonal variation.

Key words: Field evaluation, Pigeonpea, Seed colour, Seed size, Somaclonal variation, Yield.

Considerable research has been done and substantial amount of literature have been published on somaclonal variation in various crops where in most cases, the *in-vitro* cell cultures displayed significant abnormalities in the form of chromosomal aberrations, chlorophyll deficiency, fertility alterations, and many other unwanted morphological characters (Bairu *et al*. 2010). However, some somaclonal variants derived from single gene mutation with large recognizable effects may be useful in the genetic improvement programmes. Although such variants for plant height (Larkin *et al*. 1984), seed colour (George and Rao 1983), and herbicide resistance (Chaleff and Ray 1984) have been reported, but their exploitation in genetic enhancement of yield has been rather limited. Studies have also been conducted to ascertain the use of somaclonal variants in plant breeding. In pigeonpea, Reddy and Rao (1975) reported significant *in-vivo* somatic variation for maturity, seed size, pod size and stem pigmentation in pigeonpea. The first attempt to create genetic variability through *in-vitro* culture of somatic cells in pigeonpea was made by Chintapalli *et al*. (1997). They regenerated plants from the cotyledonary explants of pigeonpea and reported significant variation for plant height, seed size, seed colour and insect resistance in R_2 and R_3 generations. The present study reports the results of field evaluation of the selected somaclones for some important agronomic and quality traits.

MATERIALS AND METHODS

Chintapalli *et al*. (1997) regenerated pigeonpea plants from cotyledonary explants and studied *in-vitro* somaclonal variation under pot culture experiments for some morphological characters in R_1 and R_2 generations. Field grown R_3 population segregated for flower colour, leaf shape, flowering habit, pollen fertility, pod borer damage, seed size, and seed colour. At maturity, over 100 plants were selected and transferred to the pigeonpea breeding unit of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for further utilization in breeding programme. At R_4 generation, advanced through pedigree selection, the single plant progenies were sown in Alfisols at the onset of rainy season where a number of promising single plants were visually selected at maturity mainly for more number of pods/plant (as an indicator of more seed yield), seed size, and seed colour. These selections were further advanced for three generations using pedigree method of breeding. In 2000 rainy season, 15 promising progenies were bulk harvested and kept under cold storage.

In 2006, those lines were rejuvenated for their field evaluation for various agronomic characters along with the parent variety 'ICPL 87' in two replications during the rainy seasons of 2007 and 2008. A basal doze of di-ammonium phosphate was applied @ 100 kg/ha to ensure good crop growth and full expression of characters. The seeds were sown in Alfisols on ridges at 60 cm × 10 cm spacing in randomized complete block design (RCBD) at the onset of rains. Each plot consisted of four rows of four metre length. To control the weeds, a pre-emergence herbicide Fluchloralin was applied @ 2 l/ha that was followed by three hand weedings. The experiments were irrigated as and when required. One spray of Monocrotophos 36% EC was applied @ of 1 l/ha to control *Maruca vitrata* damage at early flowering stage. This was followed by two sprays of Methomyl @ 1 l/ha and one spray of Endosulfan 35% EC @ 2 l/ ha for controlling *Helicoverpa armigera* damage during podding stage. Data were recorded for days to maturity, plant height, grain yield, seeds/pod, seed size, and seed colour. Net plots, measuring 9.12 m^2 were harvested at maturity.

Two samples from eight inbreds and the control were also assessed for their major cooking quality parameters. The cooking quality analyses were performed using decorticated dry split peas. For protein estimation, random samples of 70 mg *dal* were placed in a digestion tube. One auto-tablet (Kjel-tab) and 3 ml of H_2SO_4 - H_3PO_4 mixture 95 parts conc. H_2SO_4, 5 parts of 85% H_3PO_4 (v/v) were added to the digestion tube and the samples digested at 370°C for 1 h. After cooling, distilled water was added to bring its volume to 75 ml. An aliquot from the digested sample was used for nitrogen estimation in Technicon Auto Analyzer and the nitrogen values were converted into protein by multiplying by a factor of 6.25. The cooking time was determined by boiling the samples (10.0±0.5 g) in 50 ml of distilled water in a BD-20 heating block digester (Tecator, Sweden). To determine cooking time, the boiled samples were examined at one minute intervals for their softness by pressing them between the forefinger and the thumb. For water absorption study, the samples (5.0 g ± 0.5 g) were boiled for 20 min in 35 ml of distilled water in BD-20 block digester. After boiling, excess water was decanted and the samples weighed. The amount of water absorbed by the samples was calculated and the results were expressed as increase in weight per gram of sample. The percentage of solids dispersed into the cooking water was determined by boiling the samples (5.0 + 0.5 g) for 20 min. The boiled material was passed through a 20 mesh sieve. After thorough washing, the residue was dried at 110°C for 3 h and the loss in sample weight was calculated and expressed as percentage of solids dispersed in the cooking water.

RESULTS AND DISCUSSION

In general, growth habit of the somaclonal variants was found to be more or less similar to that of the parent variety 'ICPL 87'. Pooled analysis of the data showed that the differences amongst the genotypes over two years were significant for days to maturity, plant height, 100-seed weight and seed yield (Table 1). Variation between two years was significant for days to maturity, 100-seed weight and seed yield. The interactions between genotypes × years, however, were found to be significant only for maturity. This suggested that in both the years, the performance of genotypes followed more or less similar trends in respect of seed yield, plant height, seed size and seeds/pod.

The genetic variation created through *in-vitro* culture for grain yield was significant in both the years (Table 1). In 2007, the trial mean yield (1254.5 kg/ha) was low as compared to that of 2008 (2222.7 kg/ha). On average over the two years, the variant line, 'ICPL 99073' was found to be the best performer with 2226 kg/ha yield and recorded 25.3% advantage in yield over the parent variety 'ICPL 87' (1777 kg/ha). This line produced maximum yield (2861 kg/ha) in 2008, and was among the top performers in 2007. Overall, the genetic variation for days to maturity was large where the parent variety 'ICPL 87' matured in 124 days, while 'ICPL 99068' matured earlier (118 days) than the control (Table 2). Since the earliness in pigeonpea is controlled by more than one partially dominant gene (Saxena and Sharma 1990), the deviations observed between 'ICPL 87' and the somaclonal variants probably represent mutational changes in one or two gene loci, which contributed towards earliness in 'ICPL 99068'. Some somaclonal lines such as 'ICPL 99073', 'ICPL 99070' and 'ICPL 99066' were taller than the parent variety (Table 2). In pigeonpea, the inheritance of plant height has been reported to be complex and quantitative in nature. Its expression is often complicated and masked by thermo- and photo-period sensitivity (Byth *et al*. 1981, Wallis *et al*. 1981), and therefore, no attempt was made to interpret the present results in terms of induced variation at genetic level.

In pigeonpea, the characters such as seed size and seed colour are very important from marketing point of view. In comparison to the traditional small brown seeded varieties, large white seeded types are considered to be premium and fetch about 15 - 20% higher price. The popular variety 'ICPL

Table 1. Mean squares for different characters recorded at ICRISAT in 2007 and 2008 rainy seasons

Source	Seed yield (g)	Days to maturity (no.)	Plant height (cm)	Seeds/pod (no.)	100-seed weight (g)
Pooled analysis					
Years	18000365**	5880.0**	596.3	2.07	34.24**
Entries	345945*	39.9*	409.3**	0.14	3.98**
Year x Entries	192941	57.0**	72.8	0.2	0.64
Pooled error	172102	18.9	67.3	0.12	0.25
2007					
Replications	216089	0.01	63.3	0.38	0.45
Entries	483508*	79.79	234.5	0.19	2.63**
Error	205593	35.00	103.3	0.10	0.45
2008					
Replications	135479	10.94	84.90	0.46*	0.13
Entries	355377*	17.19	247.47**	0.14	1.99**
Error	155356	10.94	49.34	0.12	0.14

*, **: Significant at P=0.05 and 0.01, respectively

87' has brown seeds with 100-seed weight of 11.1 g. In the present study, more than 50% of the variants showed gains of 13.6 to 21.8% in their seed size (Table 2). The largest seeds (13.3 g/100-seeds) were produced by 'ICPL 99073'. This line also produced high seed yield (2226 kg/ha). Since small seed size is dominant over large seeds (Singh and Pandey 1974), the observed variation for large seeds could be the result of specific mutational events occurring from dominant to recessive form of alleles. The most common seed coat colour in pigeonpea is brown, and it is dominant over white seed. Only one or two recessive genes are known to control the expression of white seed (Patil 1970, Singh 1971, Deokar *et al*. 1972). In the present study, 8 (out of the 15 lines evaluated) recorded a change in seed colour from brown to white (Fig. 1), emphasising a change that was induced through point mutations from dominant to recessive form of alleles. It is evident that the dominant genes, governing brown coat colour are prone to such mutagenic forces. Chintapalli *et al*. (1997) also reported the presence of white seeded mutants in some of the R_2 explants and they attributed it to the presence of some transposable elements, which were activated during the process of tissue culture process. They also postulated the presence of definite genes for white seed colour and high seed mass in the adapted genetic background of 'ICPL 87'. The present data also showed that the increases in seed size of pigeonpea were not restricted to any specific seed colour. Ryan *et al*. (1987) recorded significant gains in seed size in wheat that was induced by somaclonal changes. Morden *et al*. (1989) and Baillie *et al*. (1992), on the contrary, reported no such increases among somaclonal variants for kernel weight in barley.

Somaclonal induced resistance to *Fusarium* wilt has been reported in tomato (Shahin and Spivey 1986) and Medicago (Hartman *et al*. 1984). Also, Krishnamurthy and Tlaskal (1974) identified virus resistant somaclones in sugarcane. Pigeonpea variety 'ICPL 87' is known to be tolerant to diseases like *Fusarium* wilt and sterility mosaic virus (Saxena *et al*. 1989). In the present study, most of the mutant lines were found more or less similar to the parent variety in their disease reaction (Table 3). The limited observations, recorded in the present study in pigeonpea, suggested that *in-vitro* mutations for resistance to the two diseases were ineffective.

The assessment of cooking quality parameters of any newly developed variety is essential, particularly in view of their potential adoption. In the present study, the variant inbreds were compared with the control cultivar 'ICPL 87' for protein content, cooking time, water absorption, and solid dispersal and for each trait a limited variation (Table 3) was observed among the test lines and the mutants were more or less similar to the parent 'ICPL 87'. This indicated that in this material the somaclonal variations did not induce any significant change in the quality parameters studied and there will be no concern in their marketing and utilization. In wheat, however, Ryan *et al*. (1987) reported significant increase in protein content through induction of somaclonal variation.

Among the tested somaclonal variants of pigeonpea, 'ICPL 99073' was identified as the best as it had large white seeds, besides 25.2% greater yield than the parent variety 'ICPL 87'. This line along with some other selections such as 'ICPL 99070', 'ICPL 99072' and 'ICPL 99073' holds promise in parts of India and southern and eastern Africa, where pigeonpea varieties with white bold seeds generally fetch premium. Some high yielding somaclonal variants were also selected in oats by Dahleen *et al*. (1991). Since the phenology of these lines matches well with that of the parent cultivar, these appear to be good candidates to replace the 25-year-old

Table 2. Performance of somaclonal variants at Patancheru during 2007 and 2008 rainy seasons

		Days to Mature			Plant height (cm)			Seeds /Pod			100 seed mass (g)			Grain Yield (Kg/ha)		
S. N.	ICPL No.	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean
1	99066	115	130	123	142	147	145	4.1	4.9	4.5	13.3	11.5	12.4	1638	2134	1886
2	99068	105	130	118	130	137	134	4.1	4.3	4.2	13	12.1	12.5	1314	2449	1882
3	99069	120	130	125	142	132	137	4.6	4.6	4.6	13.2	12.8	13	1314	1835	1575
4	99070	110	130	120	145	140	143	4.2	4.7	4.5	13.3	11.2	12.2	1530	2710	2120
5	99071	118	133	126	148	135	142	4.1	4.4	4.3	13.7	11.3	12.5	861	2277	1569
6	99072	110	137	124	150	143	147	4.4	4.6	4.5	13.7	12.1	12.9	1429	2830	2130
7	99073	110	137	124	160	142	151	4.3	4.8	4.5	14.1	12.6	13.3	1590	2861	2226
8	99074	118	133	126	135	123	129	4.2	4.6	4.4	13	10.8	11.9	756	2177	1467
9	99075	120	130	125	118	123	121	4.3	5.2	4.7	10.1	10.3	10.2	875	1945	1410
10	99076	120	130	125	135	128	132	4.9	4.6	4.7	10.8	9.8	10.3	1448	1979	1714
11	99077	112	130	121	128	113	121	4.5	4.9	4.7	11.9	11.4	11.6	1527	1792	1660
12	99078	115	130	123	130	120	125	4.9	4.7	4.8	13.2	11.7	12.5	1222	2161	1692
13	99079	118	132	125	125	130	128	4.6	4.7	4.6	12.4	11.5	11.9	973	2122	1548
14	99080	118	133	116	130	130	130	3.6	5	4.3	14.1	11.9	13	1093	2426	1760
15	99081	120	130	125	140	130	135	4.6	4.7	4.6	12.3	10.8	11.5	906	1907	1407
16	87 (parent)	118	130	124	130	125	128	4.6	4.9.	4.8	11.7	10.4	11	1596	1958	1777
	Mean	114.1	131.6	124.6	136.7	131.1	133.4	4.4	4.7	4.59	12.7	11.4	11.9	1255	2223	1835
	CV (%)	5.2	2.5	3.5	7.4	5.4	6.2	7.5	7.5	7.5	5.3	3.3	4.1	36.1	17.7	22.6
	CD (P=0.05)	9.01	4.08	7.51	15.44	8.80	14.37	0.43	0.43	0.64	1.07	0.43	0.86	687.70	488	725.23

variety 'ICPL 87'. However, more multi-location trials would be necessary to confirm their performances across locations over years. White bold seeded pigeonpea cultivars are also preferred as fresh vegetable. Since most present day vegetable cultivars are of long-duration with a short fruiting period (Saxena *et al*. 2010), the adoption of short-duration vegetable types will be a boon to farmers because of their perennial growth habit and longer fruiting period. In such lines the green pod harvest commences early in the season and allows multiple harvests for a longer duration to help farmers in generating more income.

Tissue culture is a potential tool in creating genetic variation through *in-vitro* selection of somaclones for targeted traits. The lines derived from somaclonal variations for distinct morphological traits represent certain genetic changes in near isogenic background, expectedly, arising due to point mutations. Such genetic materials are ideal for studying genetic nature of the characters in detail. Ullrich *et al*. (1991) reported the selection of important somaclonal variants, largely arising due to *in-vitro* genetic mutations. Such mutations can either be from dominant to recessive gene form or *vice versa*. Zehr *et al*. (1987) and Lee and Philips (1987) reported that most of the culture-induced phenotypes were inherited as single recessive gene. Brettel *et al*. (1986) conducted molecular studies in a somaclonal variant of maize (*Zea mays*) and reported that a single base substitution was responsible for this variability. Ryan *et al*. (1987) and Larkin *et al*. (1984), however, reported the presence of both monogenic dominant as well as recessive forms of mutations among the tissue culture derived explants of wheat (*Triticum aestivum*). Often, the minor gene mutations can also occur, but their effects cannot be detected, particularly those with complex

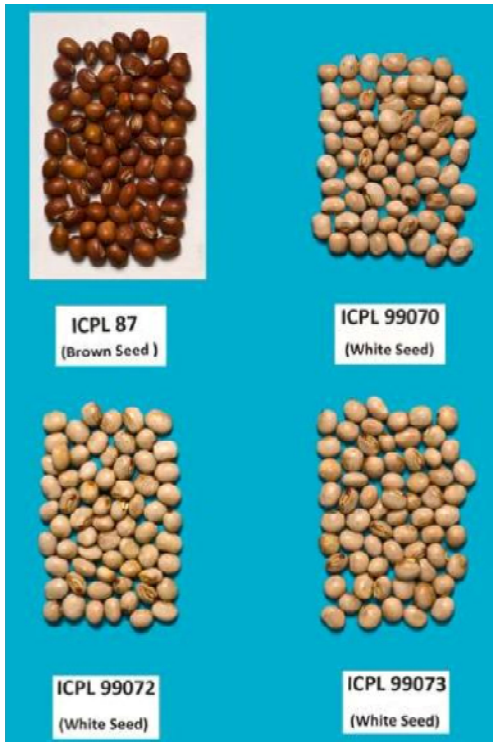

Fig 1. Seed colour and size of parent var. 'ICPL 87' (top left) and three large white seeded somaclonal mutants in pigeonpea.

inheritance. If the changes were brought due to mutation for a trait in more than one line, then such useful genes can be pooled together for greater phenotypic effects and stability by pyramiding those minor genes into a single genotype. The mutagenic changes occurring at the level of single DNA nucleotide that occur in a coding region can also result in

Table 3. Some quality parameters and disease reaction of a few promising somaclones

Designation ICPL No.	Wilt* (%)	SM* (%)	Seed colour	Protein (%)	Cooking time (min)	Water absorption (g/g)	Solid dispersion (%)
99068	33.5	9.5	white	22.1	16	1.32	25.3
99069	22.5	21.5	white	20.6	21	1.31	36.4
99070	41.5	25.0	white	20.6	20	1.29	34.2
99072	23.5	3.0	white	19.8	22	1.34	34.5
99073	21.5	3.5	white	20.3	22	1.33	34.3
99078	19.5	6.0	brown	19.9	20	1.33	41.1
99079	21.5	5.5	brown	20.0	18	1.31	33.4
99080	12.5	3.0	brown	20.2	20	1.32	37.3
99066	22.5	12.5	white	-	-	-	-
99071	27.5	7.0	white	-	-	-	-
99074	21.0	8.0	white	-	-	-	-
99075	31.0	13.5	brown	-	-	-	-
99076	47.5	10.5	brown	-	-	-	-
99077	23.5	12.5	brown	-	-	-	-
99081	34.0	21.0	brown	-	-	-	-
87 (parent)	20.5	4.0	brown	20.7	18	1.37	37.8
ICP 2376 (C)	82.0	0.0	white	-	-	-	-
ICP 8863 (C)	0.0	100.0	brown	-	-	-	-

ICP 2376 wilt susceptible check, ICP 8863 Sterility mosaic susceptible check, *Mean values recorded for tolerance to disease evaluated under sick plot

additional genetic variability. In addition, the point mutations arising from base substitutions also provide a good source for generating variability. Generally, recessive mutations are not detected in the *in-vitro* regenerated plants, but often expressed in their progenies. The experiments of Chintapalli *et al*. (1997) showed that *in-vitro* environment is mutagenic for pigeonpea and the callus-derived somaclones show both negative as well as positive variation. The deleterious negative mutants can be eliminated in early generation of selection. Tissue culture generates novel variants that is sometime difficult to breed through traditional methods. The successful utilization of somaclonal variability depends primarily on the genotype, its systematic evaluation and selection and its judicious utilization in breeding for genetic enhancement. It is also true that the tissue culture induced variations are free from various complex legal ownership issues and certain socio-ethical problems as encountered by the genetically modified crops.

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