African Journal of Biotechnology Vol. 7 (5), pp. 587-590, 4 March, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Evaluation of the shoot regeneration response in tissue culture of pigeonpea (*Cajanus cajan* [L.] Millsp.) varieties adapted to eastern and southern Africa

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Accepted 3 January, 2008

Seven varieties of pigeonpea (*Cajanus cajan* [L.] Millsp.) of varying growth durations and adapted to a wide range of environments across eastern and southern Africa were evaluated for their shoot regeneration response in tissue culture. On a standardized shoot regeneration medium, the short duration varieties (ICPV 88091 and ICPV 86012) generally responded faster and better than the medium duration (ICEAP 00554 and ICEAP 00557) and long duration (ICEAP 00020, ICEAP 00040 and ICEAP 00053) varieties. However, all the tested varieties produced healthy rooted plants *in vitro* that could be transferred to the greenhouse where they exhibited normal growth, flowering and viable seed set. This study established the basis for genetic engineering of African pigeonpea varieties.

Key words: Shoot regeneration, pigeonpea, *Cajanus cajan*, African varieties.

INTRODUCTION

Pigeonpea (*Cajanus cajan* [L.] Millsp.) is an important grain legume of the semi-arid tropics (Nene et al., 1990). In Africa, it provides protein-rich food, firewood and income for resource poor smallholder farmers (Ritchie et al., 2000). The planting of pigeonpea also replenishes soil nutrients and controls soil erosion (ICRISAT, 1998). Unfortunately, several diseases and insect pests cause major losses in Africa. A major pigeonpea disease, Fusarium wilt, is being controlled through conventional plant breeding (Gwata et al., 2006, Silim et al., 2005). However, control of *Helicoverpa armigera*, a pest that causes major yield losses, through conventional plant breeding has not been possible due to lack of genetic sources of resistance. Since the pod damage or seed loss greatly reduce the yield of pigeonpea, such pod- and seed-damaging insects are considered the most important pests in pigeonpea cultivation (Minja et al., 1999).

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The best options currently available for control of insect pests are through use of chemical insecticides that are expensive and not affordable for most farmers in Africa. Genetic engineering provides unique possibilities to incorporate genes from unrelated species from both eukaryote and prokaryote sources into pigeonpea (Sharma et al., 2004). A number of tissue culture protocols have been published for pigeonpea of which the ones reporting direct organogenesis proved to be most promising for its genetic engineering (Yadav and Padjmaja, 2003; Misra 2002; Geetha et al., 1998; George and Eapen, 1994; Kumar et al., 1983). In addition, a genotype-independent regeneration and *Agrobacterium*-mediated transformation protocol from leaf explants has recently been reported for Asian varieties of pigeonpea (Dayal et al., 2003) that has been shown to be efficient for the genetic transformation of pigeonpea (Sharma et al., 2006) and was used to introduce the rice chitinase gene into this crop (Kumar et al., 2004; Sharma et al., 2006).

To date, all tissue culture and genetic engineering research in pigeonpea have been in Asia and on varieties

Table 1. Summary of the results of eight pigeonpea varieties evaluated for shoot regeneration response in tissue culture. Results represent a total of 8 experiments. In each experiment, 70 seeds of each variety were placed on the seed germination medium and leaf explants were taken from the two cotyledons. Shoots developed in the petiolar region.

Variety (duration)	Germination frequency (% of total no of seeds)	Leaf explants	% explants with shoots after 4 weeks	% of shoots to RIM	No of rooted plants (% of shoots on RIM)
ICPL 88039 (short)	28	308	46	78	13 (12)
ICPL 87091 (short)	23	262	63	73	7 (6)
ICPL 86012 (short)	27	301	48	70	2 (2)
ICEAP 00554 (med)	28	307	64	19	1 (3)
ICEAP 00557 (med)	13	142	43	36	1 (5)
ICEAP 00020 (long)	14	158	44	46	2 (6)
ICEAP 00040 (long)	8	97	53	39	1 (5)
ICEAP 00053 (long)	11	127	77	24	3 (13)

of Asian origin. To apply the tools of genetic engineering for the improvement of pigeonpea in Africa, we evaluated the shoot regeneration response of seven varieties that are adapted to a wide range of environments in eastern and southern Africa.

MATERIALS AND METHODS

The shoot regeneration protocol for pigeonpea, for varieties grown in that was Asian that was developed previously at ICRISAT, Patancheru (Dayal et al., 2003; Sharma et al., 2006) was applied to evaluate the regeneration response, through direct organogenesis from the petiolar region of leaf explants of seven pigeonpea varieties grown in Africa. These included long duration varieties ICEAP 00020, ICEAP 00040 and ICEAP 00053; medium duration varieties ICEAP 00554 and ICEAP 00557 and short duration varieties ICPL 86012 and ICPL 87091. ICPL 88039 (Dayal et al., 2003) was included as a control. Seeds were surface sterilized with 30% (v/v) commercial bleach (equivalent to 1% NaOCl) for 30 min followed by thorough washing (3 to 4 times) with sterile distilled water. The seeds were germinated *in vitro* on medium containing MS (Murashige and Skoog, 1962) basal salts (Ducheffa), 3% (w/v) sugar and solidified with 0.8% (w/v) Difcobacto agar. The preparation of explants, culture medium and conditions were the same as reported by Dayal et al. (2003). Shoot induction medium consisted of MS supple-mented with 5 µM BA, 5 µM, kinetin, 3% (w/v) sugar and 0.8% (w/v) agar (SIM). Well-developed shoots (3 cm tall) were transferred to shoot elongation medium (SEM) consisting of germination medium supplemented with 0.58 µM GA_3. Elongated shoots were exposed to a pulse treatment of dipping for 2 min in 11.4 µM IAA, prior to culture on root induction medium (RIM) consisting of MS supple-mented with 1% sugar. Rooted plants were transferred to pots containing a mixture of sand and vermiculite (1:1) and maintained in a greenhouse up to plant maturity and seed collection.

RESULTS AND DISCUSSION

All seven varieties evaluated in this study responded well compared to the control (ICPL 88039) and the results are summarised in Table 1. The number of leaf explants obtained varied significantly among the varieties due to the variation observed in the seed germination of individual varieties. Short duration varieties (ICPL 88039, ICPL 87091 and ICPL 86012) generally germinated better than medium and long duration varieties, with ICEAP 00040 exhibiting the lowest seed germination frequency (Table 1). Germination of individual varieties also tended to vary between experiments. In some experiments, only 2 to 6 explants could be obtained from 70 seeds for the long duration varieties ICEAP 00020, ICEAP 00040 and ICEAP 00053 as well as the medium duration variety, ICEAP 00557. Therefore, care should be taken when applying this protocol for transformation studies to ensure that adequate numbers of leaf explants are obtained. This can be done by ensuring that only good quality seed and optimum germination temperatures for the respective varieties are used.

The leaf explants formed multiple shoots within 7 to 14 days following culture on SIM. Shoots that formed were generally strong and healthy and up to 78% of these were transferred to rooting medium after 4 weeks when they were about 3 cm tall (Table 1). Short duration varieties (ICPL 88091, ICPL 88039 and ICPL 86012) in general responded faster in tissue culture and larger percentages of shoots from these varieties could be transferred to RIM. For all the tested varieties, but mostly for the medium and long duration ones, healthy shoots often were contaminated in the RIM, probably due to latent endogenous contaminants. This accounted for the low number of rooted plants (Table 1). It is, therefore, recommended that seeds used as starting material should be obtained from healthy plants, preferably grown in a greenhouse or screenhouse where they are protected from the bacterial and fungal pathogens which can be transmitted through seeds.

A small number of rooted plants from all the seven varieties as well as the control were acclimatized in a greenhouse to complete the reproductive cycle. In general, the acclimatized plants transferred easily to soil and short duration varieties flowered within two months and successfully set viable seeds (Figure 1).

Conclusion

In conclusion, it was found that the regeneration protocol

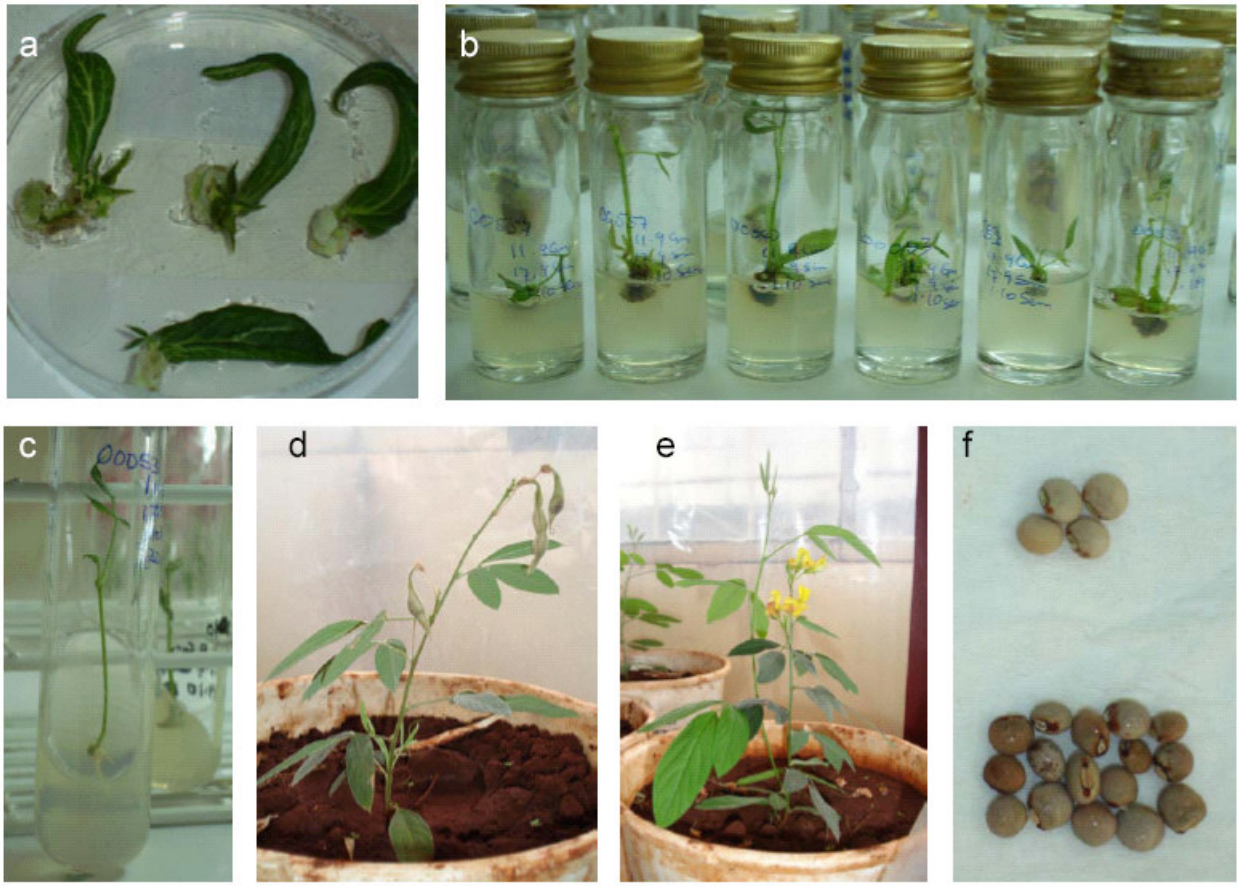

Figure 1. The stages of regeneration of pigeonpeas adapted to Africa: (a) Cotyledon explants of ICEAP 00554 with shoot buds and shoots regenerating in the petiolar region. (b) Shoot buds elongating in SEM. (c) A shoot of ICEAP 00053 with adventitious roots. (d and e) Acclimatized plants of ICPL 87091 in the greenhouse exhibiting normal flowers and seed pods. (f) Fertile seeds of ICPL 87091 produced from tissue culture regenerated plants (top) compared to seeds produced in the field (bottom).

of Dayal et al. (2003) is applicable to pigeonpea varieties developed by ICRISAT for Africa. Although, short duration varieties responded better in terms of the number of explants that produced shoots and subsequently rooted plants, all the varieties could be regenerated. For genetic engineering of pigeonpea, short duration varieties would be the genotypes of choice, although characteristics such as existing resistance traits, adaptability across a wide range of environments and crossing compatibility with various duration types should also be taken into consideration.

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