

Effect of 6-BA on the Plant Regeneration *via* Organogenesis from Cotyledonary Node of Cowpea (*Vigna unguiculata* L. Walp)

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Abstract

The present study compares effects of different concentrations of 6-BA on regeneration from cotyledonary node explants of cowpea (*Vigna unguiculata* L. Walp). The seeds were inoculated on MSB₅ medium [Murashige and Skoog (1962) salts and Gamborg B₅ vitamins (1968)] containing different concentrations (0, 1, 2, 3, 4, 5 mg/L) of 6-BA for 4 days. The cotyledonary node explants with one cotyledon excised from 4-day-old seedlings, placed *in vitro* on MSB₅ medium containing 6-BA at different dose (0, 0.5, 1.0, 1.5 mg/L) for shoot induction and elongation. Best response in terms of shoot number and shoot length were obtained with explants derived from seedling preconditioning with 3mg/L 6-BA followed by the induction and elongation stage pretreated with 0.5mg/L 6-BA. The elongated shoots were rooted on MSB₅ medium without hormone.

Keywords: cowpea, plant regeneration, cotyledonary node, organogenesis, 6-BA

Abbreviations: 6-BA: 6-Benzylaminopurine

1. Introduction

Cowpea (*Vigna unguiculata* L.) is widely grown in Africa, Latin America, Southeast Asia and southwestern regions of North America, and is a major source of high-quality dietary protein and energy for local people. It plays an important role in the lives of millions of people in developing countries of Africa and Asia. In spite of the great importance of this crop, its productivity is low, which is mainly limited by the damage caused by biotic and abiotic stresses (Singh et al., 1997). In addition, limited genetic diversity in cowpea breeding programs is of special concern because cowpea appears to have lower inherent genetic diversity than other cultivated crops as a result of a hypothesized single domestication event (Fang et al., 2007). Although some resistance genes to insect pests and fungi have been identified in some IITA cowpea varieties and other closely related *Vigna* species (Latunde-Dada et al., 1990), the attempts using conventional breeding methods to introduce the resistance genes into the cultivated cowpea have made little progress for the strong hybrid incompatibility. Hence, genetic engineering approaches stand out as the most effective alternative strategy to overcome the production constraints (Zaidi et al., 2005). An effective and rapid regeneration protocol is essential for genetic transformation. Plant regeneration of cowpea *via* organogenesis has been achieved from epicotyls, hypocotyls, primary leaves, cotyledons, cotyledonary nodes, shoot tips, plumular apices and shoot meristem. Of these, cotyledonary node explants seemed the most responsive for the induction of multiple shoots, which was appropriate to *agrobacterium*-mediated transformation (Chaudhury et al., 2007; Raji et al., 2008; Solleti et al., 2008a, 2008b; Adesoye et al., 2010).

Previous work has studied the effect of varied hormones used together on the regeneration of cowpea. But the regeneration of cowpea via cotyledonary node uses 6-BA alone has not been explored. The aim of this paper is to explore the effect of 6-BA on different stages of regeneration of cowpea, to provide a theoretical and technical basis for rapid propagation.

2. Materials and Methods

2.1 Plant Materials and Seeds Preconditioning

Mature seeds of cv. Cheng-jiang VII of cowpea was obtained from the Research Institute of Horticulture, Academy of Chengdu Agriculture and Forestry Science, Chengdu, China. The seeds were soaked with 70% ethanol for 1 min, surface-sterilized with 0.2% (w/v) HgCl₂ for 5 min, followed by rinsed five times with sterile

distilled water and blotted with sterilized filter papers. Then the seeds were cultured on MSB₅ medium supplemented with 6-BA at different concentrations (0, 1, 2, 3, 4, 5 mg/L) for 4 days.

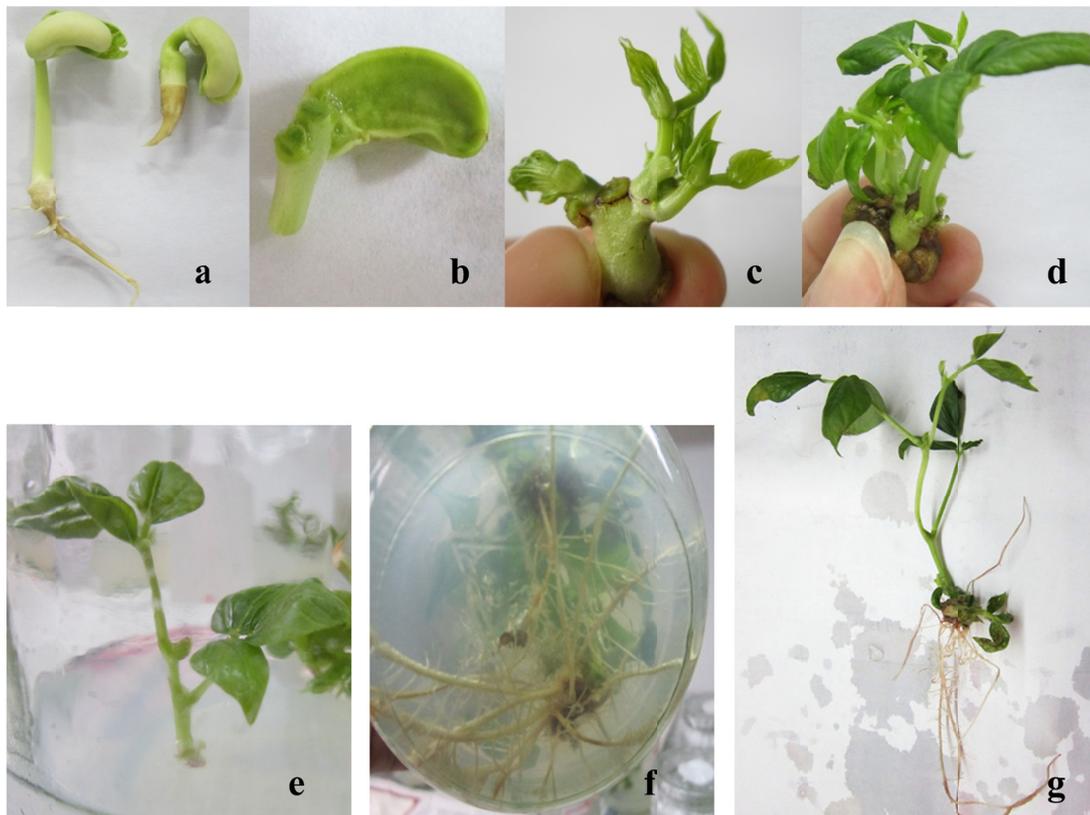


Figure 1. Regeneration system from cotyledonary node explants of cowpea. (a) Seedling preconditioning with 6-BA (right) and seedling without preconditioning (left). (b) Cotyledonary node explants removing one cotyledon and cutting both the epicotyls and hypocotyls. (c) and (d) Explants excised from seedling preconditioning with 3 mg/L 6-BA, followed by shoot induction and elongation on MSB₅ + 0.5 mg/L 6-BA for two weeks (c) and four weeks (d). (e) Elongated shoots were separated and transferred to hormone-free MSB₅ medium for rooting. (f) Elongated shoots forming roots on hormone-free MSB₅ medium. (g) Rooted plantlet

2.2 Shoot Induction and Elongation

The cotyledonary node explants excised from 4-day-old seedlings were cultured in a vertical upright position with the hypocotyls end slightly embedded in MSB₅ medium supplemented with various concentrations of 6-BA (0, 0.5, 1.0, 1.5 mg/l). The explants were excised by removing one cotyledon and cutting both the epicotyls and hypocotyls approximately 2 mm above and 3-5 mm below the nodal point (Figure 1b). And the initial axillary buds were also removed. After 2 weeks of culture (Figure 1c), the multiple shoots were removed from the explants and transferred to fresh medium with the same concentrations of 6-BA for subculture for another 2 weeks (Figure 1d).

2.3 Rooting and Acclimatization

Regenerated shoots were separated and transferred to hormone-free MSB₅ medium for rooting (Figure 1e). After 2 weeks of culture, the rooted plantlets (Figure 1g) were washed in running tap water and transferred to pots containing sterilized soil, green manure and vermiculite at 1:1:1 ratio. Each pot was covered with transparent polyethylene bags to maintain adequate humidity during the first few days. Subsequently, the bags were removed and the plants were allowed to grow at room temperature with 50% relative humidity.

2.4 Culture Medium and Conditions

MSB₅ medium [Murashige and Skoog (1962) salts and Gamborg B₅ vitamins (1968)] supplemented with 3% (w/v) sucrose and 0.6% (w/v) agar was used throughout this study. The pH of the medium was adjusted to 5.8

with 0.1 N NaOH or HCl before autoclaving at 121°C for 15 min. All the cultures were maintained at 26±2°C temperature with 16 h light photoperiod. The experiment started from mid-August and finished in mid to end of October in 2012.

2.5 Data Collection and Statistical Analysis

The length and the number of adventitious buds were recorded after 2 weeks of culture on the shoot induction and elongation medium. The date of the shoots began to take roots were also recorded. The experiments were arranged to repeat thrice with 20 replicates per treatment. The data were determined by analysis of variance and the significant difference between the means were compared using Duncan's new multiple range method with the help of statistical software DPS.

3. Result and Discussion

3.1 Effect of 6-BA Preconditioning on Seed Germinating

Cowpea is recalcitrant to regeneration from shoot proliferation and genetic manipulation (Dita et al., 2006). The regenerative competence could be increased *via* seedling preconditioning using high dose of cytokinin because of its promoting in cell division.

In this study, the seeds cultured on the medium containing 6-BA grew obviously stronger than those cultured on hormone-free medium. The differences are expressed in following aspects made up of dramatic enlarged primary leaves, stubby hypocotyls, hyperplastic region of the cotyledonary node, and thick and short roots (Figure 1a). This conclusion was similar to some other scholars (Bakshi et al., 2012; Tang et al., 2012). The effect was significantly more pronounced when the concentration of 6-BA was up to 3 mg/L. Looking just from morphological terms, the seedlings showed no obvious differences at high dose (3,4,5 mg/L) of 6-BA.

3.2 Effect of 6-BA on Shoot Induction, Elongation and Rooting

Table1. Effect of different concentration of 6-BA on shoot regeneration from cotyledonary node explants of cowpea following culture on MSB₅ medium for two weeks

Seedling preconditioning	Concentration of 6-BA (mg/L)		Mean number of shoots per explant	Mean shoot length (cm)
	Shoot induction and elongation			
0	0		1.82d	2.83ab
	0.5		3.68c	2.17abcd
	1		4.59ab	1.50cde
	1.5		4.44bc	0.93e
1	0		1.90d	2.93ab
	0.5		4.93ab	2.50abc
	1		5.30ab	2.33abcd
	1.5		4.97ab	1.86bcde
2	0		2.27d	2.53abc
	0.5		5.17ab	1.97bcde
	1		4.89ab	2.17abcd
	1.5		5.32ab	1.47cde
3	0		2.23d	3.20a
	0.5		5.20ab	2.80ab
	1		5.10ab	2.40abcd
	1.5		5.13ab	1.53cde
4	0		2.00d	3.10a
	0.5		5.30ab	2.22abcd
	1		5.41ab	1.93bcde
	1.5		5.15ab	1.37de
5	0		2.13d	3.10a
	0.5		5.53a	2.49abc
	1		5.42ab	1.88bcde
	1.5		5.33ab	1.60cde

Values represent means. Means having the same letters are not significantly different according to Duncan's multiple range test at $P = 0.05$.

At the stage of shoot induction and elongation, more adventitious buds were observed on the media supplement with 6-BA compared with that of control. When the concentration of 6-BA was 0.5 mg/L, 1.0 mg/L and 1.5 mg/L, the number of shoots per explant displayed increasing but the distinction was not very significant (Table 1). This might be because higher concentration of 6-BA precondition at the stage of seedlings made a large impact on the following stage (Brar et al., 1999; Le et al., 2002; Raveendar et al., 2009). The shoot length was decreased with the concentration of 6-BA increasing, which was in agreement with the research of pioneers (Diallo et al., 2008; Aasim et al., 2009; Tang et al., 2012). Besides, it is important to note that when the concentration of 6-BA was 1.5 mg/L, abnormal morphology of shoots would be observed. The stems grew and bend downwards, the leaves were shrunken. And the abnormal shoots were difficult to elongate.

After induction and elongation, the regenerated shoots were removed on MSB₅ medium without hormone for rooting. More than 95% of the regenerative shoots could produce roots. But the time at the beginning of forming roots were different. If the concentration of 6-BA was higher at the stages of induction and elongation, it was difficult to produce roots, which manifested as it would take a longer time to start rooting. The experiment showed that the optimal concentration of 6-BA promoted the propagation of adventitious buds, but inhibit both the shoot elongation and rhizogenesis.

In the present investigation, 3 mg/L 6-BA preconditioning during the seedlings and 0.5 mg/L 6-BA at the induction and elongation stages was the best concentration to induce adventitious buds and to elongate comprehensively considered efficacy and cost.

References

- Aasim, M., Khawar, K. M., & Ozcan, S. (2009). *In vitro* micropropagation from plumular apices of Turkish cowpea (*Vigna unguiculata* L.) cultivar Akkiz. *Scientia Horticulturae*, 122, 468-471. <http://dx.doi.org/10.1016/j.scienta.2009.05.023>
- Adesoye, A. I., Togun, A. O., & Machuka, J. (2010). Transformation of cowpea (*Vigna unguiculata* L. Walp.) by *Agrobacterium* infiltration. *Journal of Applied Biosciences*, 30, 1845-1860.
- Bakshi, S., Roy, N. K., & Sahoo, L. (2012). Seedling preconditioning in thidiazuron enhances axillary shoot proliferation and recovery of transgenic cowpea plants. *Plant Cell, Tissue and Organ Culture*, 110, 77-91. <http://dx.doi.org/10.1007/s11240-012-0132-y>
- Brar, M. S., Al-Khayri, J. M., Morelock, T. E., & Anderson, E. J. (1999). Genotypic response of cowpea *Vigna unguiculata* (L.) to *in vitro* regeneration from cotyledon explants. *In Vitro Cellular & Developmental Biology-Plant*, 35, 8-12. <http://dx.doi.org/10.1007/s11627-999-0002-4>
- Chaudhury, D., Madanpotra, S., Jaiwal, R., Saini, R., Kumar, A. P., & Jaiwal, P. K. (2007). *Agrobacterium tumefaciens*-mediated high frequency genetic transformation of an Indian cowpea (*Vigna unguiculata* L. Walp.) cultivar and transmission of transgenes into progeny. *Plant Science*, 172, 692-700. <http://dx.doi.org/10.1016/j.plantsci.2006.11.009>
- Diallo, M. S., Ndiaye, A., Sagna, M., & Gassama-Dia, Y. K. (2008). Plants regeneration from African cowpea variety (*Vigna unguiculata* L. Walp.). *African Journal of Biotechnology*, 7, 2828-2833.
- Dita, M. A., Rispaill, N., Prats, E., Rubiales, D., & Singh, K. B. (2006). Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. *Euphytica*, 147, 1-24. <http://dx.doi.org/10.1007/s10681-006-6156-9>
- Fang, J., Chao, C. T., Roberts, P. A., & Ehlers, J. D. (2007). Genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] in four West African and USA breeding programs as determined by AFLP analysis. *Genetic Resources and Crop Evolution*, 54(6), 1197-1209. <http://dx.doi.org/10.1007/s10722-006-9101-9>
- Gamborg, O. L., Miller, R. A., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research*, 50, 151-158. [http://dx.doi.org/10.1016/0014-4827\(68\)90403-5](http://dx.doi.org/10.1016/0014-4827(68)90403-5)
- Latunde-Dada, A. O. (1990). Genetic manipulation of the cowpea (*Vigna unguiculata* [L.] Walp.) for enhanced resistance to fungal pathogens and insect pests. *Advances in Agronomy*, 44, 133-154.
- Le, B. V., Carvalho, M. H., Zuily-Fodil, Y., Thi, A. T., & Van, K. T. H. (2002). Direct whole plant regeneration of cowpea [*Vigna unguiculata* (L.) Walp] from cotyledonary node thin cell layer explants. *Journal of Plant Physiology*, 159, 1255-1258. <http://dx.doi.org/10.1078/0176-1617-00789>
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue

- cultures. *Physiologia Plantarum*, 15, 473-497. <http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Raji, A. A. J., Oriero, E., Odeseye, B., Odunlami, T., & Ingelbrecht, I. L. (2008). Plant regeneration and *Agrobacterium*-mediated transformation of African cowpea [*Vigna unguiculata* (L.) Walp] genotypes using embryonic axis explants. *Journal of Food, Agriculture & Environment*, 6, 350-356.
- Raveendar, S., Premkumar, A., Sasikumar, S., Ignacimuthu, S., & Agastian, P. (2009). Development of a rapid, highly efficient system of organogenesis in cowpea *Vigna unguiculata* (L.) Walp. *South African Journal of Botany*, 75, 17-21. <http://dx.doi.org/10.1016/j.sajb.2008.05.009>
- Singh, B. B., Chambliss, O. L., & Sharma, B. (1997). Recent advances in cowpea breeding. *Advances in Cowpea Research* (pp. 30-50).
- Solleti, S. K., Bakshi, S., & Sahoo, L. (2008a). Additional virulence genes in conjunction with efficient selection scheme and compatible culture regime enhance recovery of stable transgenic plants in cowpea via *Agrobacterium tumefaciens*-mediated transformation. *Journal of Biotechnology*, 135, 97-104. <http://dx.doi.org/10.1016/j.jbiotec.2008.02.008>
- Solleti, S. K., Bakshi, S., Purkayastha, J., Panda, S. K., & Sahoo, L. (2008b). Transgenic cowpea (*Vigna unguiculata*) seeds expressing a bean α -amylase inhibitor 1 confer resistance to storage pests, bruchid beetles. *Plant Cell Reports*, 27, 1841-1850. <http://dx.doi.org/10.1007/s00299-008-0606-x>
- Tang, Y., Chen, L., Li, X. M., Li, J., Luo, Q., Lai, J., & Li, H. X. (2012). Effect of culture conditions on the plant regeneration via organogenesis from cotyledonary node of cowpea (*Vigna unguiculata* L. Walp). *African Journal of Biotechnology*, 11(14), 3270-3275. <http://dx.doi.org/10.5897/AJB11.3214>
- Zaidi, M. A., Mohammadi, M., Postel, S., Masson, L., & Altosaar, I. (2005). The Bt gene *cry2Aa2* driven by a tissue specific ST-LS1 promoter from potato effectively controls *Heliothis virescens*. *Transgenic Research*, 14, 289-298. <http://dx.doi.org/10.1007/s11248-004-7714-3>