

Full Length Research Paper

***In vitro* Shoot Induction and Regeneration of Cowpea (*Vigna unguiculata*) from Embryonic Axis Explants**

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The experiment was conducted to test the *in vitro* regeneration efficiency of cowpea shoots via embryonic axes explants and the effect of benzyl amino purine (BAP) concentration on shoot formation. A total of 600 explants (embryonic axes) were plated from which 237 shoots were regenerated. The average regeneration ratio was 39% and the number of shoot per explant obtained stood at an average of 6.0. This indicates likelihood that higher regeneration percent and number of shoots per explant could be possible as more proficiency is attained in the use of the protocol. The role of the growth regulator, benzyl amino purine (BAP), as a shoot growth inducing factor and modifier in cowpea *in vitro* regeneration was demonstrated in the experiment. The protocol used in the experiment gave promising results for the efficient regeneration and subsequent genetic transformation of cowpea.

Key words: Cowpea, *in vitro*, regeneration ratio, benzyl amino purine (BAP), embryonic axes explants.

INTRODUCTION

Cowpea is an important crop for farmers in much of the West African region, especially in the dry savannas. Estimates of world hectareage of cowpea are in the range of 12.5 million with about 8 million in West Africa, the majority being in Nigeria and Niger (FAO, 2000). Cowpea faces several abiotic and biotic stresses for which conventional breeding alone can not provide ultimate solutions. For example, grain yield losses are mainly due to damage caused by insect pests and diseases, as well as abiotic stresses such as heat and drought (Singh et al., 1997). Plant Biotechnology and genetic engineering approaches offer alternative ways of overcoming these stresses. In addition to direct transfer of genes of agronomic interest, genetic transformation techniques can be used to answer many basic questions pertaining to cowpea biology such as understanding of gene function and regulation of physiological and developmental processes (Gelevin, 1998). These benefits require the development of reliable, efficient and reproducible methods of cowpea genetic transformation.

Although legumes are considered “recalcitrant” to regeneration and transformation, routine protocols for obtaining stable transformants are now available for the major grain legumes such as the common bean (*Phaseolus vulgaris*), soybean (*Glycine max*), pea (*Pisum sativum*), peanut (*Arachis hypogea*), and alfalfa (*Medicago sativa*) as well as the model legume, barrel medic (*Medicago truncata*) (Russell et al., 1993). However, development of tractable gene transfer systems in cowpea has been impeded by several constraints. Cowpea is not of major economic importance to the most technologically advanced countries in North America and Europe. The production areas of this crop are mainly tropical Africa, Asia, and Latin America where technical expertise and infrastructure for biotechnology research are either lacking or poor. Therefore, comparatively little work has been done to develop and optimize regeneration and transformation procedures, in comparison to temperate crops that are of economic importance to the North, including recalcitrant cereals (Komari et al., 1998).

Routine application of molecular improvement independent of chosen method of transformation is still hampered by the lack of readily available and highly efficient and long-term regenerable cell and tissue culture systems. Furthermore, recent reports have indicated that

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Table 1. Cowpea regeneration efficiency via embryonic axes.

Experiment No.	Initial Number of Explants	Number of Shoots Regenerated ^o	Regeneration Ratio (%)	Average Number of Shoots per Explant
1	150	38	25	6
2	150	53	35	5
3	150	75	50	5
4	150	71	47	6
Total	600	237		

^oPercent regeneration determined at sixth sub-culture (64 days old)

transgenic plants regenerated through a more-or-less long-term callus phase have an increased risk of somaclonal variation, problems in transgene inheritance and stability of expression (Bregitzer and Tonks, 2003). Therefore, the objective of this paper is to report results from the experiment conducted to test the *in vitro* regeneration efficiency of cowpea shoots via embryonic axes explants and the effect of benzyl amino purine (BAP) on shoot formation of embryonic axes explants of cowpea.

MATERIALS AND METHODS

Four regeneration experiments were conducted and cowpea variety IT86D1010 was used as a model genotype. For each experiment, surface sterilization of seeds was achieved by soaking 48 g of seeds in 0.3% hypochlorite solution for one hour. After thorough shaking, the solution was poured off and the seeds rinsed thoroughly thrice with sterile distilled water. The sterilized seeds were spread on sterile paper towel ready for cutting to excise the embryo from the cotyledon. Seeds were split-opened with embryo attached to one cotyledon using a sterile forceps and scalpel. About 2 mm from the tip of the radicle is cut-off and the plumule removed just at the nodal point. The decapitated embryos were severed from the attaching second cotyledon. Thereafter, the decapitated embryos were placed horizontally on shoot induction (MS + 2 mg/L BAP) medium. Twenty five explants were plated per Petri dish. The number of explants per experiment was 150. All activities were carried out inside a vertical laminar airflow cabinet. Plated Petri dishes were covered with parafilm and kept in the culture room.

Sub-culturing: Six transfers of surviving explants to another medium were conducted for each regeneration experiment. The first subculture, which was carried out four days after plating, lead to three transfers of explants to shoot proliferation (MS + 0.5 mg/L BAP) medium for ten days for each transfer, while subsequent three transfers of surviving explants to shoot elongation (MS + 0.1 mg/L BAP) medium was conducted. Thereafter, surviving explants had their radicle end reduced to remain only the greenish region with nodal points. They are then plated with 75% insertion in the medium. At each transfer, multiple shoots were carved and plated on fresh culture medium. All cultures were maintained at 26°C under a 16/18 h (light/dark) photoperiod with light supplied by cool-white day light fluorescent lights 28 - 36 $\mu\text{molm}^{-2}\text{s}^{-1}$. Different media were prepared as follows:

Shoot induction medium: The following reagents were dissolved in 1000 mL distilled water; 30 g/L sucrose, 4.43 g/L MS basal salt, 10 mL/L B5 vitamin, 8 g/L agar and 2 mg/L BAP. The pH of the mixture was adjusted to 5.8 (Murashige and Skoog, 1962). The mixture was autoclaved for 15 min at 121°C.

Shoot proliferation medium: The following reagents were dissolved in 1000 mL distilled water: 30 g/L sucrose, 4.43 g/L MS basal salt, 10 mL/L B5 vitamin, 8 g/L agar and 0.5 mg/L BAP. The pH of the mixture was adjusted to 5.8. The mixture was autoclaved for 15 min at 121°C.

Shoot elongation medium: The following were dissolved in 1000 mL distilled water: 30 g/L sucrose, 4.43 g/L MS basal salt, 10 mL/L B5 vitamin, 8 g/L agar and 0.1 mg/L BAP. The pH of the mixture was adjusted to 5.8. The mixture was autoclaved for 15 min at 121°C.

RESULTS AND DISCUSSION

A total of 600 explants (embryonic axes) were plated from which 237 shoots were regenerated (Table 1, Figure 2). The average regeneration ratio was 39% and the number of shoot per explant obtained stood at 6.0. In a similar experiment, Chaudhary et al. (2007) reported an average regeneration ratio of 80% and average number of shoots per explant of 6.3. Experiment 3 had the highest percent of shoot regeneration (50%) while experiment 1 had the lowest (25%) (Table 1). Number of shoots regenerated per explant was highest (6) in experiment 1 and 4, respectively.

Placing embryonic axes on shoot induction medium (2 mg/L BAP) actually induced shoot formation (Figure 1). About 85% of plated axes with greenish nodal region were sub-cultured. Reducing BAP concentration to 0.5 mg/L BAP in shoot proliferation, lead to emergence of shoot from nodal point of surviving explants. Similar observation was made by Obembe et al. (2000) who reported that direct organogenesis from cotyledons, cotyledonary nodes, epicotyls, and primary leaves cultured on Murashige and Skoog medium containing optimal levels of BAP appear to be reproducible and hold promise for use in transformation. Not less than 60% explants produced multiple shoots at this stage (Figure 1). Sub-culturing every ten days on shoot elongation medium (0.1 mg/L BAP) did not produce much elongation of shoots. Problems encountered at this stage included browning of shoots, oxidative burst and dead cells around multiple shoots. Many explants were lost at the 5th transfer stage in all the experiments. This contributed to moderate regeneration efficiency of the experiment.

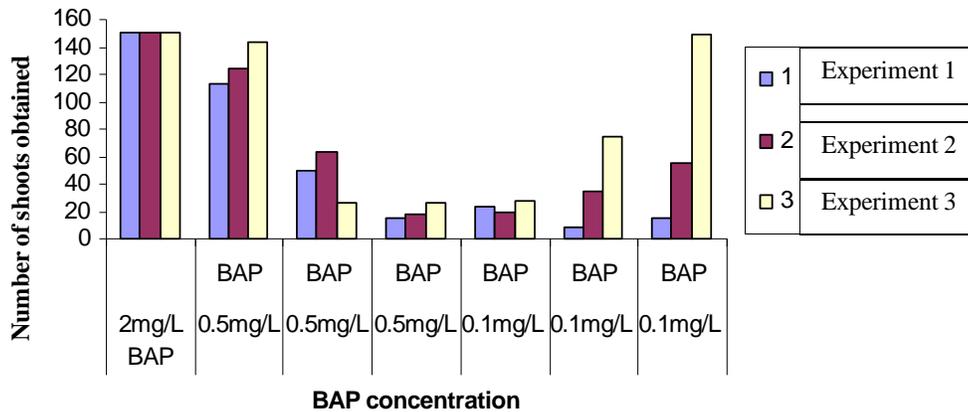


Figure 1. Effect of BAP concentration on shoot formation from embryonic axes explants of cowpea.

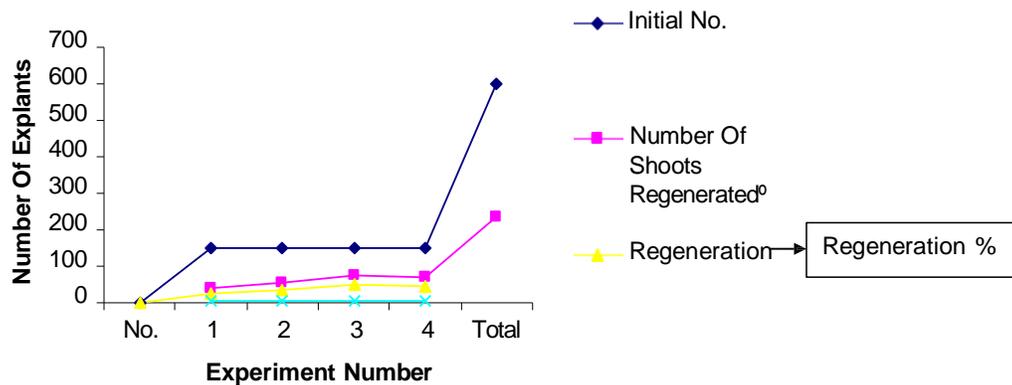


Figure 2. Cowpea regeneration efficiency via embryonic axes explants.

Conclusion

The percent regeneration and number of regenerated shoots per explant obtained in this experiment stood at 39% and 6 respectively. This indicates likelihood that higher regeneration percent and number of shoots per explant could be possible as more experiments are performed and more proficiency is attained in the use of the protocol. The role of the growth regulator, BAP, as a shoot growth inducing factor and modifier in cowpea *in vitro* regeneration was demonstrated in the experiment. The problem of contaminants was minimally observed and frizzled out as more experiments were being conducted. However, problems of oxidative burst and dead and brown tissues persisted throughout. The protocol used in the experiment has potential of efficient regeneration and genetic transformation of cowpea with improvements and modifications.

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