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MEDIA OPTIMIZATION FOR THE GROWTH AND EXPRESSION OF PROTEIN YIELD IN CULTURE RECALCITRANT PLANT *CICER ARIETINUM L.* (CHICKPEA)

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ABSTRACT
Beans and legumes are inexpensive and a common food all over the world. Typically high in fibre, calcium, and iron, beans and legumes are also a great source of protein. In the perspective of increasing protein production in the world, strategies allowing maintained yield should be preferred. Legumes generally and chickpea (*Cicer arietinum*) particularly is supposed to be recalcitrant and difficult to manipulate in *in vitro* cultures. However, selection of right explants and appropriate range of different concentrations of auxin and cytokinin resulted in appreciable good response in embryonic development. Present investigation attempted to obtain plant regeneration from callus induced from different types of explants and identified the role of commercially available high yielding growth regulator milieu in culture medium on the callus induction and protein yield ability. An efficient and reliable plant regeneration protocol for *in vitro* micro-propagation of *Cicer arietinum* was established. It is concluded that immature embryo is the best source of explants and high callus induction frequency was obtained on multiplication medium. Present studies showed that callus induced on medium with 1.0 µg/ml IAA and Kinetin and a maximum of protein concentration was observed in embryonic callus grown on regeneration medium with IAA (10.68 µg/ml) and Kinetin (6.7 µg/ml). This protocol might be promising towards future research in *in vitro* micro-propagation of *Cicer arietinum* and can be applied to large scale multiplication of selected germplasm of *Cicer arietinum* having high protein yielding properties.

KEYWORDS: explant, callusing medium, multiplication medium, protein yield.

INTRODUCTION

Associated with cereals, legumes constitute the main component of traditional dishes throughout the world (Graham, 2003). Despite growing demand and high-yield potential, chickpea productivity is very low. Several borer, and abiotic such as drought, salinity and low temperature, constraints are major factors for lower chickpea production (Bliss *et al.*, 1973 and Frimpong *et al.*, 2009). Modern breeding technologies with biotechnological techniques are required to increase the productivity. One of the areas of these techniques is micro-propagation of plants. The aim of this technique is a fast production of a great number of genetically identical plants from a highly valuable mother plant (Neumann, 2006). These plants can be either directly sold on the market for planting, used for breeding purposes, for gene technology or the technique is used as a method for basic science studies. Because we think that the main challenge for grain legumes in human nutrition is linked to their role as a source of protein, the main focus of this study was the improvement of protein content, bio-availability, and nutritional quality of chick-pea.

MATERIALS & METHODS

Various micro-propagation techniques have facilitated greatly the development of improved varieties in several crop species. The studies in chickpea are still in its infancy. In order to devise the best strategy to improve protein content in chickpea, we tried to optimize media to culture *Cicer arietinum*. The cell suspension culture of *Cicer arietinum* was maintained in the Murashige & Skoog medium supplemented with Indole Acetic Acid and Kinetin. Cells (1.5 g fresh wt) were transferred to l00-ml Erlenmeyer flasks containing 25 ml medium, grown in continuous light (600 lux) at 100 rev. /min and 23^0C, harvested on a Buchner funnel and immediately frozen in -80^0C. The frozen cells were allowed to thaw in an equal amount (v/w) of ice-cold 0.25 M Tris-maleate buffer (pH 7.7), containing 5 mM mercaptoethanol and grounded in a mortar with some quartz sand (Schulze *et al.*, 2006). Various combinations of IAA and Kinetin were included in the study. The homogenate was strained through cheese cloth and centrifuged at 12000 x g for 10 min. The supernatant was saturated to 80% with $(NH_4)_2SO_4$, the precipitate recovered by a 10 min centrifugation at 19000 x g and dissolved in a small volume of the extraction buffer. Protein concentrations in cell-free extracts were determined by the standard method (Lowry *et al*., 1951).

RESULT & DISCUSSION

Legume seeds provide an exceptionally varied nutrient profile, including proteins, fibres, vitamins and minerals (Mitchell *et al*., 2009). Nitrogen that is used by the young seedling during germination is stored in the seed in the

form of storage proteins. Seeds contain from 16% to 50% of protein and provide one third of all dietary protein nitrogen (Graham and Vance, 2003). Anticipating the increasing demand for protein food sources, the Protein Advisory Group of the United Nations has identified the improvement of legumes as a critically important area of research. The protein-rich legumes as a complement to cereals make one of the best solutions to protein-calorie malnutrition, particularly in developing countries. The carbon energy supply that is needed upon germination is stored in grain legume seeds either mainly in the form of oil (soybean, groundnut), or as starch (common bean, pea, fababean, lentil, chickpea, cowpea, mungbean) (Hedley, 2001).

Scientists have been improving plants by changing their genetic makeup since the late 1800s. Typically, this has been accomplished through crossbreeding and hybridization, in which two related plants are cross-fertilized and the resulting offspring have characteristics of both parent plants. In the breeding process, however, many undesirable traits often can appear in addition to the desirable ones. Some of those undesirable traits can be eliminated through additional breeding, which is time consuming. Breeders can then further select and reproduce the offspring that have the desired traits. Many of the foods that are already common in our diet are obtained from plant varieties that were developed using conventional genetic techniques of breeding and selection (Hulse, 1975, Singh & Jambunatham, 1981, Cho, 2002, Guillamon, 2008).

Chickpea grows during the time of the year when many other legumes are cropped, displaying considerable drought avoidance or tolerance. Root traits are likely to be one of the most important components of draught tolerance in chickpea. Using petiole explants from transgenic plants containing the auxin responsive MAS promoter linked to the GUS reporter gene the distribution of auxin within the cultured petiole could be followed during the induction phase of somatic embryogenesis (Neumann, 2006). Micro-propagation Technique in Enhancing the Productivity of Crops has been taken up at large scale at TERI (Saxena, 2004; Kumar and Shekhawat, 2007).

It is concluded that immature embryo was the best source of explants and high callus induction frequency was obtained on multiplication medium. Present studies showed that a maximum of protein concentration is observed in embryonic callus grown on regeneration medium with IAA (10.68µg/ml) and Kinetin (6.7µg/ml) with callus induced on media with 1.0 µg/ml IAA and Kinetin, *i.e.*, in the combination of IAA-8 and Kinetin-8 (Table 1).

Legumes generally and chickpea particularly is supposed to be recalcitrant and difficult to manipulate in in vitro cultures. However, selection of right explants and appropriate range of different concentrations of auxin and cytokinin in this study resulted in appreciable good response in embryogenic development. This protocol can be used for further improvement of chickpea crop by micro-propagation.

TABLE 1: Absorbance of embryonic protein at 660nm measured by Folin Lowry method showing effect of Auxin and cytokinin at various concentrations in embryo culture medium

S.N.	Growth regulator	24-05-2012 Absorbance for auxin	24-05-2012 Absorbance for cytokinin	31-05-2012 Absorbance for auxin	31-05-2012 Absorbance for cytokinin	07-06-2012 Absorbance for auxin	07-06-2012 Absorbance for cytokinin
1	IAA1/Kinetin1	0.14	0.0	0.24	0.26	0.30	0.02
2	IAA2/Kinetin2	0.12	0.09	0.2	0.16	0.28	0.10
3	IAA3/Kinetin3	0.17	0.26	0.03	0.14	0.30	0.07
4	IAA4/Kinetin4	0.22	0.28	0.19	0.12	0.21	0.06
5	IAA5/Kinetin5	0.14	0.31	0.12	0.09	0.27	0.02
6	IAA6/Kinetin6	0.14	0.22	0.1	0.03	0.22	0.05
7	IAA7/Kinetin7	0.46	0.19	0.09	0.01	0.29	0.08
8	IAA8/Kinetin8	0.5	0.12	0.04	0.06	0.32	0.11
9	IAA9	0.59	-	0.12	-	0.31	-
10	IAA10	0.22	-	0.15	-	0.36	-

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