
PROTEIN PATTERN IN DIFFERENTIATING EXPLANTS OF CHICKPEA (CICER ARIETINUM L.)

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SUMMARY

Protein pattern of differentiating and non-differentiating calli were studied using SDS-PAGE in two varieties of chickpea viz. BG 267 (kabuli) and BG 362 (desi). Both MS and B5 media, supplemented with growth regulators and silver nitrate, were used for this purpose. A protein band of molecular weight around 50,000 was identified in the differentiating calli of the kabuli variety which was absent in the non-differentiating calli. MS medium was found suitable for multiple shoot development in epicotyl tissue whereas, B5 media was found suitable for shoot bud production in hypocotyl (H) and cotyledonary node (CN) explants in both the varieties.

Key words: Chickpea, Differentiation, Protein, SDS-PAGE.

INTRODUCTION

Chickpea (Cicer arietinum L.) is one of the major grain legumes of India. Efforts are being made to produce transgenic plants in this crop for pest and disease resistance (Fontana et al., 1993) using established regeneration protocols (Barna and Wakhlu, 1994; Polisetty et al., 1997). However, it has not been successful because of the extreme recalcitrance nature of this crop. An understanding of the processes involved in differentiation at the molecular level may help to overcome the barriers. Growth and differentiation involve the metabolism of various proteins at different stages. Identification of such proteins associated with differentiation and dedifferentiation is important to elucidate the biochemical and molecular mechanisms underlying the process. This may help to overcome the difficulties encountered in regeneration of otherwise recalcitrant crop species. In pea, the protein pattern shown by nodular, yellowish, embryogenic calli was remarkably different from the compact, non-embryogenic calli (Sturm and Jacobsen, 1987). Similarly, variations in protein pattern in differentiating and non-differentiating tissues was reported earlier in Oryza sativa L. (Chen and Luthe, 1987), in barley and sugarcane (Ramagopal 1989, 1994) and in Nicotiana tabacum (Garcia et al., 1992). Therefore, the present study was undertaken to find out the differences in the electrophoretic patterns of proteins between differentiating and non-differentiating explants of chickpea.

MATERIALS AND METHODS

Two varieties of chickpea viz. BG 362 (desi) and BG 267 (kabuli) were used for the present study. For media preparation, growing the seedlings and inoculation and maintenance of cultures under aseptic conditions, the methods of Chandra et al. (1993) was followed. Four day old hypocotyl (H) and cotyledonary node (CN), and seven day old epicotyl, each about five mm long, were obtained from the aseptically grown seedlings and used as explants. In the first set of experiments, B5 medium (Gamborg et al., 1968) supplemented with or without silver nitrate (AgNO3) was used for the inoculation of H and CN explants of both the varieties. Silver nitrate supplemented medium is termed as differentiating medium.
whereas the one without AgNO₃ is termed as non-differentiating medium. In the second set of experiments, both MS (Murashige and Skoog, 1962) and B5 media were used to study the influence of medium on the protein patterns of differentiating explants. Both the media were supplemented with or without silver nitrate as specified earlier. Epicotyl explants, which are more regenerative than both H and CN, of only the desi variety (BG 362) were used in this experiment.

To find out the changes in total protein profiles in the differentiating and non-differentiating calli, protein analysis was done by one dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) using the LaemmliBuffer system (Laemmli, 1970). For all the samples studied, soluble protein of the calli was extracted in 0.6 M Tris buffer (pH 6.8) at 4°C. For this, one gram of sample was macerated with four ml of extraction buffer in a pestle and mortar kept in ice. Protein estimation was carried out following the Bradford (1976) method.

RESULTS AND DISCUSSION

The role of ethylene in inhibiting differentiation was confirmed by the application of AgNO₃, an action inhibitor of ethylene, in the media which resulted in the production of shoot buds in H (Plate 1). Similarly, callus developed from CN also developed shoot buds in the presence of AgNO₃ in the media (Plate 2). Thus, the media used in the present study in which silver nitrate was supplemented is called as the differentiating media.

Hypocotyl and cotyledonary node explants of the kabuli variety (BG 267) usually produced a loose, green, non-differentiating callus. But when silver nitrate (25μM) was supplemented to the media, these explants produced a compact, brown callus which differentiated into shoot buds (Plates 1 & 2). The differentiating callus produced a distinct protein band of molecular weight around 50,000 (lanes 3 & 5, Plate 3) which was absent in the non-differentiating calli (lanes 4 & 6, Plate 3). This confirms with the results of Garica et al. (1992) that new proteins are synthesized at the time of differentiation. Similar


Moreover, when a lower concentration of silver nitrate (12 μM) was used in the media, the calli of both the explants did not differentiate into shoot buds. The intensity

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Plate 3. Electrophoretic patterns of total proteins of 60d old differentiating (D) and non-differentiating (ND) calli of H and CN explants of chickpea (Cicer aritinum). Variety: BG 267. AgNO3: 12.5μM (Lane 1), 25μM (Lane 3 & 5). M: Molecular markers. Lanes: 1-4, H (1 & 3D; 2&4, ND), 5-6, NC (5, D; 6, ND).

Plate 4. Electrophoretic patterns of total proteins of 60d old differentiating (D) and non-differentiating (ND) calli of H, CN and epicotyl explants of chickpea (Cicer aritinum). Variety: BG 362. AgNO3: 15μM (Lanes 2, 4, & 6), 50μM (Lane 8). Non-Differentiating calli, Lanes: 1&9 (Ha), 3(CN), 5&7 (Epicotyl). Differentiating calli, Lanes: 2&10(H), 4(CN), 6&8 (Epicotyl). (Note : Lanes 7 & 8 show protein bands of epicotyl explants grown in MS media).

of the protein bands produced by this callus was less (lanes 1 & 2, plate 3) as compared to those produced by the differentiating callus in the presence of 25 μM AgNO3 (lanes 3 & 4, plate 3). Thus, the increase in the amount of proteins with an increase in the concentration of AgNO3 is associated with the production of shoot buds. However, reports are lacking on the role of cytokinins, supplemented in the medium along with silver nitrate, in the induction of polypeptide metabolism associated with shoot bud differentiation especially in recalcitrant crops like chickpea.

The protein patterns obtained in the desi variety BG 362 is completely different from those obtained in the kabuli variety BG 267. In this variety, the differentiating nodular callus (in the presence of 25 μM AgNO3) of H and CN explants (lanes 2 & 4 respectively, plate 4) produced less number of polypeptide bands than the non-differentiating green calli (lanes 1 & 3, plate 4). This difference was more clearly visible in CN explants (lanes 3 & 4, plate 4). Further, the intensity of the protein bands were also more in this variety as compared to the kabuli variety. Thus in BG 362, less number of polypeptides were recorded in the explants from the differentiating media and also from a more differentiating tissue like CN, when compared to that recorded in BG 267 which was more responsive to the differentiation treatment. This observation shows the varietal differences in the protein pattern of differentiating and non-differentiating calli. Earlier reports in chickpea (Chandra et al., 1993, 1996, 1997/98) and sunflower (Chirby et al., 1991) indicated that the CN explants were more regenerative than the H explant. In this variety, among the explants, the intensity of the protein bands was more in H than in CN indicating the presence of higher amount of protein in H. It seems that these proteins are inhibitory to both growth and differentiation and exclusion of these proteins may probably result in differentiation of the H explant (Ramagopal, 1994). On the other hand, Leshem and Sussex (1990) could not find any relationship between the appearance of proteins and growth of cotyledonary node explants in melon.
Since the epicotyl explant is more regenerative than both H and CN, it was also used in the present study. Differences in protein bands were seen between the calli grown in MS medium (lanes 7 & 8, plate 4) and those grown in B5 media (lanes 5 & 6, plate 4). Differences were also observed between control and silver nitrate treatment. The protein bands with their molecular weight around 14,000 were not clearly visible in the epicotyl explants in B5 medium supplemented with silver nitrate (lane 6, Plate 4). The callus from the epicotyl issue produced more number of protein bands than H and CN. The epicotyl explant produced multiple shoots in MS media supplemented with growth regulators and silver nitrate. The number and intensity of protein bands in the MS media were more than that in the B5 media. This shows that MS medium, containing both the forms of nitrogen (ammonium and nitrate), induces new proteins in higher amounts whereas such proteins were not induced in the B5 media, which contains nitrogen mainly in the form of nitrate and with an amount about only half of that present in MS media.

For the epicotyl explant, MS media was found to be more suitable than B5 media. For H and CN, B5 media was more suitable for growth and differentiation (Guru, 1997) as it was also suitable for the production of buds in the presence of AgNO₃ in these explants. In terms of total nitrogen concentration, MS media contains double the amount of nitrogen as compared to that of the B5 media. Probably the formation of multiple shoots and their subsequent growth requires more amount of total nitrogen and in both forms. On the other hand, shoot bud differentiation in H and CN explants seems to require less amount of total nitrogen, that too in nitrate from only as evident from their positive response in B5 media (Niedz. 1994). The total amount of nitrogen in B5 media is lesser as compared to the MS media. As both H and CN explants are metabolically less active than the epicotyl explant and also due to their proximity to the root system, their N requirement in terms of nitrate can be explained. Thus, the present study exhibited the influence of genotype on the proteins related to differentiation and the role of these proteins in morphogenesis in chickpea. Further identification of these proteins will be useful in characterizing the biochemical and molecular aspects of growth and differentiation of different explants grown in vitro and their manipulation for further advantage.

REFERENCES


