Indian J. Plant Physiol., Vol. 10, No. 1, (N.S.) pp. 100-102 (Jan.-Mar., 2005)

SHORT COMMUNICATION

BANDING PATTERN OF PROTEIN SUBUNITS WITH TUBER DEVELOPMENT IN *SOLANUM TUBEROSUM* L.

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Received on 4 March, 2004; Revised on 4 April, 2005

Tubers of two developmental stages, *viz.* 45 days after planting (DAP) (>5 g) and 80 DAP (25-30 g) were collected from five Indian genotypes *viz*., JX-1, MF-1, 85-P-ll, 85-P-670 and 85-P-718 grown in field conditions during Rabi 2000-2001. The protein was extracted and electrophoresed through 12.5% acrylamide SDS gels. Results indicated that protein composition in tubers 5g at 45 DAP of two stages was quite different in 40-68 kD and 20-35 kD regions. Banding patterns in small developing tubers >5g at 45 DAP were almost uniform across the genotypes. Absence of polypeptides in 20-35 kD and presence of dark bands in 35-40 kD and below 14 kD regions in mature tubers of 25-30g weight at 80 DAP were reproducible and could be used for variability studies. Heat treatment to the crushed tubers before centrifugation resulted in destruction of protein subunits.

Key words: **Potato, protein subunits, *Solanum tuberosum.***

Occurrence of genetic variability in gene pool is important for potato improvement (Plaisted 1972, Mendoza and Haynes 1974). Potato tuber proteins have been documented for high quality and quantity (Nakasone *et al*. 1972, Stegemann *et al*. 1973, Kapoor *et al*. 1975, Racusen and Foote 1980) and suitability for studying the genetic variation through electrophoretic analysis (Desborough and Peloquin 1968, Stegemann and Loeschcke 1976, Oliver and Martinez-Zapater 1985). For variability studies the source material should contain consistent subunits. Potato tubers provide stable source of proteins but have inconsistent composition with developmental stages (Coccuci *et al.* 1972, Rosenstock and Zimmermann 1976, Snyder and Desborough 1978). If the stage of tubers for stability in soluble proteins is judged these can effectively be used for cultivar identification (Desborough 1983). Field grown tubers are suitable for electrophoretic analysis of proteins (Stegemann *et al*. 1973, Kapoor *et al*. 1975, Stegemann and Loeschcke 1976, Park *et al.* 1983) as they provide good substrate for such studies. Therefore, this investigation was done to standardize the appropriate stage of field grown tubers for protein variability studies electrophoretically.

Five Indian potato genotypes viz., MF-1, JX-1, 85-P-ll, 85-P-670 and 85-P-718 were grown in field at Vegetable Research Centre of the University during *Rabi* 2000-2001. Field grown tubers at 2 different developmental stages *i.e*., 45 (tuber weight >5 g) and 80 (tuber weight 25-30 g) days after planting (DAP) were collected for protein extraction.

About 0.5 g of tuber from each line of each stage was crushed with 500 µl of sample buffer (Laemmli 1970) without dye, supplemented with 50 µl of 10 mM phenylmethyl sulphonyl fluoride (dissolved in 50 % ethanol) at 4°C. The supernatant was separated by centrifugation at 10,000 rpm for 10 minute at 4°C. The supernatant was kept for 1 hour at room temperature and used directly for sodium dodecyl sulphate-polyacrylamide gel electrophoresis. In another method of protein extraction,

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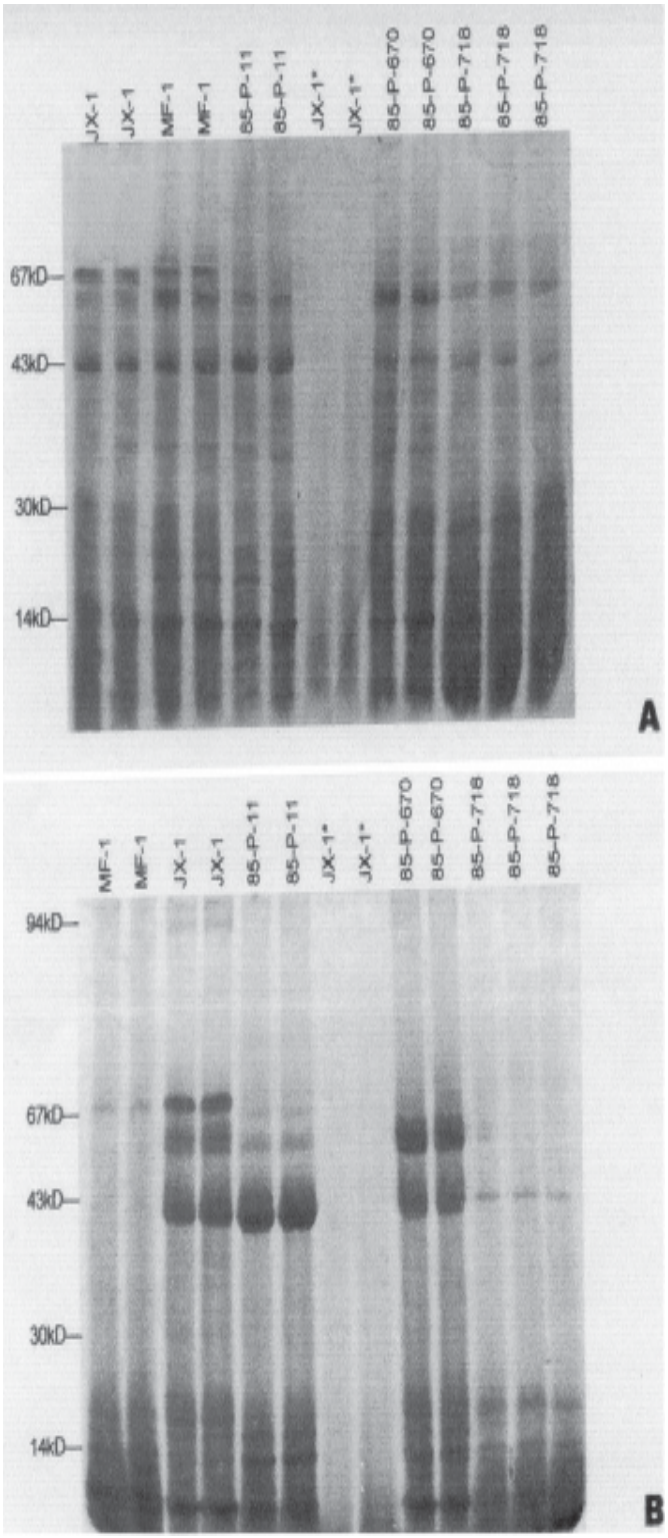

Fig 1. Banding patterns of tuber protein at two developmental stages : A. 45 days after planting (>5g); B. 80 days after planting (25-30g). * Heat treatment before centrifugation

the crushed tuber in sample buffer was heated in boiling water by dipping the base of eppendorf tubes for 3 minutes followed by centrifugation. The gels were prepared according to method described by Laemmli (1970) and Rajapakse *et al*. (1991). Protein samples of 25 µl in duplicate for each genotypes were loaded along with molecular weight marker protein consisting of phosphorylase b (94 kD), bovine serum albumin (67 kD), egg albumin (43 kD), carbonic anhydrase (30 kD) and ribonuclease a (14.0 kD) subunits. Total protein concentration was estimated for each line by the method of Bradford (1976) using bovine serum albumin (E- Merk (India) Ltd.) as standard. The quantity of protein was adjusted to 10 mg for one lane to obtain a clear separation for coomassie blue staining.

Electrophoresis was done at a constant current of 8 mA per plate for 6-7 h using electrode buffer (0.025 M Tris, 0.192 M glycine and 1.0 g l^{-1} SDS, with pH 8.4). Gels were stained with 0.2 % coomassie brilliant blue R solution (dissolved in ethanol: acetic acid: water = 45:10:45) at room temperature for 2 h. The first destaining was done using methanol: acetic acid: water (25: 10: 65) and the second with 7 % (v/v) acetic acid, both at room temperature. The intensity of colour of the bands was measured in terms of optical density (OD) at 660 nm by spectrophotometer.

The electrophoretic patterns of 5 cultivars are shown in Fig. 1. The banding pattern of tuber proteins at different developmental stages exhibited clear differences in subunits. In small developing tubers, large number of polypeptides appeared in a molecular weight range of 20-35 kD in contrast with those in mature tubers. The subunits ranging from 40-68 kD molecular weight were almost uniform across the genotype in immature tubers whereas, the protein subunits in this region were quite variable in thickness and presence or absence in mature tubers. It indicated that electrophoretic patterns of physiologically mature tubers are reliable for genetic variability studies (Snyder and Desborough 1978, Desborough 1983, Rajapakse *et al*. 1991). Rosenstock and Zimmermann (1976) has already reported twice protein content in young tubers than older ones in cultivar Saskia and it was constant when tubers reached 2-3 cm

diameter. High intensity of bands (0.8-1.0 OD) of 40 kD and those below 14 kD and absence of polypeptides in 20-35 kD region could be the most useful indicator of maturity. No band was observed beyond 68 kD in tubers of both the developmental stages. However, Snyder and Desborough (1978) indicated the presence of 80 kD subunit in tubers of 0.3 g which disappeared in tubers of 30 g. The validity of finding is corroborated by the reports of Cocucci *et al*. (1972) about rapid decrease of polysomes in young developing tubers and characterization of tuber maturation by inactivation of protein synthesis system. In another observation, when crushed tubers of cultivar JX-1 was heated in boiling water for 3 minutes it did not show any polypeptide subunit in both the developmental stages. It might be due to rapid denaturation of proteins (Paiva *et al.* 1983, Bourque *et al.* 1987, Rajapakse *et al*. 1991). Potato tuber proteins are globulins and albumins (Nakasone *et al*. 1972, Kapoor *et al*. 1975) and are quite similar to enzymes as the potato tuber is metabolically very active tissue as compared to dormant seeds. Therefore, any analytical work with potato tuber proteins should be done at lower temperatures.

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