Investigating the Potential of Petiole and Leaf blade for Callus Formation in *Solanum tuberosum* L.

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**ABSTRACT**

Different plant growth regulators (such as NAA, BAP, Kin, and 2,4-D) were used to investigate the petiole and leaf blade potential for callus formation. Best results were recorded in both NAA and 2,4-D at 1.5 mg/L. Whereas Kin gave best result at 2 mg/L for both the explants studied. In combination BAP+NAA gave better result at 2 mg/L BAP+0.5 mg/L NAA while in Kin+2,4-D better result were observed in leaf blade at 1.5 mg/L Kin +0.5 mg/L 2,4-D and in petiole at 1.5 mg/L Kin +0.5 mg/L 2,4-D.

**Key words:** Callogenesis, leaf blade, petiole, auxins, cytokinins, *Solanum tuberosum* L.

**INTRODUCTION**

Potato (*Solanum tuberosum* L.) ranks fourth in the most important food crops after maize, wheat and rice. Due to its wide adaptability potato is grown in both tropical and temperate environments up to 4000 metres (Poehlman & David, 2003). Potato grows around 18.3 million hectares with a production of 295 million tonnes. Its world's average yield is 50.5 kg/year (Prasad, 2006). Potato consumption is increasing year after year and it doubles every 10 to 15 years (CIP, 1984). In future, it will be an important food crop in the food basket of developing countries (CIP, 2005). Potato can be grown in all types of soil, except saline and alkaline soils. However, loamy soil, sandy loamy soil and organic matter enriched soil are most appropriate for the cultivation of potato crop. Moreover, it is a crop of temperate climate and is moderately tolerant to frost. Potato is one of the most economically important annual vegetable crops of family Solanaceae in Pakistan. The important potato varieties grown in Pakistan are of two types, i.e. red skin and white skin. Red skin included Desiree, Cardinal, Ultimus, Lala Faisal and Raja Symphonia while white skin included Diamant, Ajax, Patrones, Multa and Sante.

White potato, also called Irish potato, is an annual crop from the genus *Solanum* family Solanaceae (Khurana et al., 2003). *Solanum tuberosum* is a hybrid between the diploid species *S. stentotomum* and the diploid weed *S. sparsipilum* with subsequent chromosome doubling, however, over 230 wild potato species are known. The potato has a series of ploidy levels based on a haploid number of 12, ranging from diploid (2n=24) to hexaploid (6n=72), and including triploids, tetraploids, and pentaploids (Dodds & Roberts, 1995). However, the cultivated potatoes are autotetraploid (4n = 48).

The tissue culture technique is now a time-tested alternative means of plant propagation. The potato is highly responsive to many tissue culture techniques, which have been extensively applied in all aspects of production and improvement. The most significant advantage offered by micropropagation over conventional methods is that in a relatively short period of time and space a large number of plants can be produced from a single individual independently of the seasons (Smith & Drew, 1990). Micropropagation is very important for heterozygous species, such as potato, for producing uniform plants. A single explant can be multiplied into several thousands of plants in less than one year.

The present study was carried out to evaluate the callogenetic potential of leaf blade and petiole of *Solanum tuberosum* L. Two auxins and two cytokinins were used in solo and in combination to explore their impact on callogenetic response.

**MATERIALS AND METHODS**

Healthy plant shoots were selected from potato fields and brought to the laboratory. They were washed with tap water to remove the soil particles, then the shoots were surface sterilized with Mercuric Chloride (0.1% w/v) for 15 minutes.
After three washings with autoclaved distilled water, the shoots were placed in laminar air flow cabinet for excision of leaf blade and petiole. The size taken from explants was 1 cm for leaf blade and 0.5 cm for petiole. Explants were inoculated in the culture vessel with the help of forceps.

MS medium (Murashige & Skoog, 1962) supplemented with different plant growth regulators was used. The stock solutions of MS medium were prepared by mixing the inorganic and organic components. In PGR two auxins, i.e. 2,4-D (2,4-dichlorophenoxy acetic acid), and NAA (Naphthalene acetic acid) and two cytokinins, i.e. BAP (6-Benzylaminopurine), and kinetin were used in solo as well as in combination. Sucrose was added at the rate of 3.0% (w/v), pH of the medium was adjusted at 5.8 using 0.1N KOH or HCl. The medium was jelled using 0.6% agar (Bio Life). The cooked hot medium was dispensed in appropriate volumes into the culture vessels and sterilize in autoclave at 121°C, temperature 15lb/inch² pressure for 15 minutes. The laminar air flow cabinet was used for inoculation of explants. After inoculation, the vessels containing explants were placed in 25±2°C at 85% relative humidity under fluorescent light for callus induction. The results were analysed statistically in which one way ANOVA (analysis of variance) and Post Hoc test was applied.

RESULTS AND DISCUSSION

The callogenic potential of leaf blade and petiole of potato (Solanum tuberosum L.) was investigated using two auxins and two cytokinins in solo and in combination. The parameters studied included callus size, number of days for callus initiation, callus colour, texture and callusing response.

Solo Effect of Auxins

For both the explants, 0.5, 1.0, 1.5, 2.0 mg/L NAA and 2,4-D concentrations were used for callus induction. Callusing was induced at all concentrations, however, the best callus growth was observed at 1.5 mg/L NAA and 2,4-D. Petiole explant produced black-brown granular callus of 2.5±0.231 cm³ after 16 days of inoculation in NAA (Plate 2) with 81.25% response while in 2,4-D induced 2.5±0.289 yellow-brown, granular callus with 87.5% response. Dokhaniyeh et al. (2011) using the same concentration for callus induction reported production of copious callus in potato. The optimum value of leaf blade for callus size is more significant as compared to petiole, having nearly significant value at p ≤ 0.05.

Solo Effect of Cytokinins

For both the explants 0.5, 1.0, 1.5, 2.0 mg/L BAP and Kin concentrations were used for callus induction. Callusing was observed at all concentrations, however, the best callus growth was observed for petiole at 1.5 mg/L Kin and 2 mg/L BAP. Petiole explant induced callus of 1.9±0.058 cm³ in BAP, having light-yellow granular appearance and 2.25±0.029 cm³ callus in Kin (Plate 3) was produced after 18 days. It was granular, light-yellow in colour with 62.5% response. However, Sunpui & Kanchanapoom (2002) reported that by using petiole explants no callus was induced from Kin at solo form. However, in the present study leaf blade produced copious callus at 2 mg/L BAP, producing 2.21±0.020 cm³, brown, granular callus and 3.5±0.231 cm³ in Kin (Plate 4) which turns yellow-brown and granular after 16 days of inoculation with 87.5% response. Yasmin et al. (2003), using the same concentration of BAP, observed that maximum callus was induced at 5.0 mg/L and 4.0 mg/L in potato and concluded that below this concentration induction of callus is not possible. In the present study, the optimum value of petiole and leaf blade for callus size was noted significant at p ≤ 0.05.

Combined Effect of BAP & NAA

In combination, BAP and NAA were used at the strengths of 0.5, 1.0, 1.5 and 2.0 mg/L, keeping BAP constant with variable concentration of NAA. When BAP were kept constant (Figure 1-4) best result in callus production from petiole explant was observed at 1 mg/L BAP+2.0 mg/L NAA, having light-yellow, granular callus of 1.79±0.023 cm³ (Plate 5) induced after 20 days of inoculation with 68.75% response. Leaf blade gave best result at 1 mg/L BAP+1.5 mg/L NAA, producing a dark-brown,
granular callus of $2.0 \pm 0.115\, \text{cm}^3$ (Plate 6) induced after 18 days of inoculation with 50% response. Yasmin et al. (2003) studied 1 mg/L BAP+1.25 mg/L NAA showed better callus induction which is quite similar with the concentrations used in the present study. Ganzalez et al. (1999) observed that at 1 mg/L BAP+1.25 mg/L NAA concentrations successfully induced sufficient amount of callus for both the explants.

When NAA was kept constant for both the explants, the best result in petiole explant was obtained at 2 mg/L BAP+0.5 mg/L NAA, producing a yellow, friable callus of $1.9 \pm 0.058\, \text{cm}^3$ with 68.75% response after 15 days of inoculation. In leaf blade 2 mg/L BAP+0.5 mg/L NAA produced yellow, granular callus of $2.3 \pm 0.173\, \text{cm}^3$ with 75% response after 18 days of inoculation. Lima et al. (2006) also reported that BAP and NAA at these concentrations were able to induce sufficient amount of callus from the explants.

**Combined Effect of Kin & 2,4-D**

In combinations, Kin and 2,4-D were used at the strengths of 0.5, 1.0, 1.5 and 2.0 mg/L, keeping Kin constant with variable concentration of 2,4-D. When Kin was kept constant for both the explants (Fig. 5-8), best results were obtained at 0.5 mg/L Kin+1.5 mg/L 2,4-D in petiole with granular, light-yellow callus of $2.6 \pm 0.115\, \text{cm}^3$ (Plate 7) after 18 days of inoculation with 87.5% response. Leaf blade gave significant results at 2 mg/L Kin+1 mg/L 2,4-D and light-yellow granular callus of $2.8 \pm 0.173\, \text{cm}^3$ (Plate 8) induced after 15 days of inoculation with 87.5% response. Haque et al. (2009) used 0.25 mg/L Kin with 1.0, and 2.0 mg/L 2,4-D, and found it to be suitable for induction of callus. Sexene et al. (1997) observed the rapid callus growth for both explants at 0.5 mg/L Kin+2mg/L 2,4-D, and concluded that at these concentrations calli proliferated very fast and it was soft and gelatinous in texture.

When 2,4-D was kept constant for both the explants, best results were obtained in petiole at 1.5 mg/L Kin+0.5 mg/L 2,4-D, with callus size of $2.2 \pm 0.115\, \text{cm}^3$, granular, dark-yellow in colour with 87.5% response after 18 days of inoculation. In leaf blade best results were observed at 1.5 mg/L Kin+0.5 mg/L 2,4-D, producing yellow-white, granular callus of $3.5 \pm 0.289\, \text{cm}^3$ after 16 days of inoculation with 93.75% response. Haque et al. (2009) observed that leaf explant appeared to be best for callus size and weight when 1.0 mg/L 2,4-D+0.25 mg/L Kin concentration was used. Moreover, Kuehnle et al. (1992) obtained sufficient amount of callus induction at 2 mg/L 2,4-D+1 mg/L Kin.

All results were statistically analysed in one way ANOVA (analysis of variance) and Post Hoc test was applied. The optimum value of Duncan was significant for leaf blade at 0.5 mg/L 2,4-D with 0.5, 1.0, 1.5 and 2.0 mg/L Kin and 2 mg/L 2,4-D with 0.5, 1.0, 1.5 and 2.0 mg/L Kin. The results of ANOVA were significant for all concentrations in leaf blade except 1.5 mg/L BAP with different concentrations of NAA, 1mg/L Kin with different concentrations of 2,4-D, and 2,4-D with different concentrations of Kin. The results of ANOVA were significant for all concentrations of petiole except 1mg/L Kin with different concentrations of 2,4-D.
**Fig. 3:** Effect of 1.5mg/L BAP with different concentrations of NAA on leaf blade and petiole of potato

**Fig. 4:** Effect of 2 mg/L BAP with different concentrations of NAA on leaf blade and petiole of potato

**Fig. 5:** Effect of 0.5 mg/L Kin with different concentrations of 2,4-D on leaf blade and petiole of potato

**Fig. 6:** Effect of 1mg/L Kin with different concentrations of 2,4-D on leaf blade and petiole of potato

**Fig. 7:** Effect of 1.5mg/L Kin with different concentrations of 2,4-D on leaf blade and petiole of potato

**Fig. 8:** Effect of 2mg/L Kin with different concentrations of 2,4-D on leaf blade and petiole of potato
Plate 1: Response of petiole at 1.5 mg/L 2,4-D (Lateral view)

Plate 2: Response of leaf blade at 1.5 mg/L 2,4-D (Aerial view)

Plate 3: Response of petiole at 1.5 mg/L Kin (Lateral view)

Plate 4: Response of leaf blade at 2 mg/L Kin (Lateral view)

Plate 5: Response of petiole at (1 mg/L BAP + 2 mg/L mg/L NAA) (Lateral view 1x)

Plate 6: Response of leaf blade at (1 mg/L BAP + 1.5 NAA) (Lateral view 2x)

Plate 7: Response of petiole at (0.5 mg/L Kin + 1.5 mg/L 2,4-D) (Lateral view 1x)

Plate 8: Response of leaf blade at (2 mg/L Kin + 1 mg/L 2,4-D) (Lateral view 1x)
REFERENCES


