

Effects of Different Hormone Combinations and Planting Beds on Growth of Single Nodes and Plantlets Resulted from Potato Meristem Culture

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Abstract

For this study, after thermotherapy, meristems of Agria and Marfona cultivars of potato (*Solanum tuberosum* L.) were placed on filter paper bridge in liquid medium. The meristem-derived plantlets were micropropagated in solid medium by using single node cuttings. Results showed that there are significant differences between Agria and Marfona cultivars. The modified solid (MS) medium without NAA and BAP was found to be best for the formation of roots and shoots. Addition of BAP and NAA in the medium decreased shooting and rooting, respectively of single node cultures. A mixture of peat moss and sand in the ratio of 4 : 1 proved best for growing plantlets.

Introduction

Fungal and virus infections can have a dramatic effect on yield and marketable quality of a crop and also can affect adversely international distribution of potato germplasm (Towar et al. 1985).

So far about 50 virus factors have been reported on potato. Some viruses can alone decrease the yield by 40% and in combination with other viruses, the loss is 90% (Siddiqui et al. 1996).

The best control method for potato viruses is production of healthy plants from meristem culture (Bryan and Dodds 1992). Shakya et al. (1992) eliminated three potato viruses (X, Y and S) from infected tubers of cv. Cardinal by meristem culture. They showed that for producing healthy plants, small meristems (0.11 - 0.25 mm diam) had better efficiency than big meristems.

In another study, Kong et al. (1998) cultured apical meristems of four potato cultivars in modified MS solid medium with BAP, NAA and GA₃. They obtained 68.1 - 86.6% plantlets in 46 - 64 days. They showed that modified medium with NAA is the best for rapidly producing virus free plantlets.

Meristem isolation and culture on a sterile medium are delicate, therefore a few plantlets can be obtained in each replication of an experiment. For this reason, micropropagation methods are used for producing more plantlets from each meristem.

The plantlets resulted from meristem culture grew well and produced 4 - 8 lateral buds. The single node cutting culture in the solid medium, or shoot culture in liquid medium can be made for propagating them under sterile conditions.

Different factors such as type of medium, pH, sucrose concentration, type of hormone, temperature and light conditions can affect propagation and growth of plantlets from meristem culture under *in vitro* conditions.

Badawi et al. (1996) compared liquid and solid (MS) medium for single node cutting culture of Cara potato cultivar. The results showed that liquid medium did not affect the percentage of surviving cuttings, but root cutting per cent and shoot length decreased. They also studied the effects of IBA (1.0 or 2.0 mg/l), NAA + IBA (1.0 mg/l) and IBA + florigicidin (1.0 mg IBA + 160 mg florigicidin) on rooting of nodal cuttings. They found that the modified medium (MS) with 2 mg/l of IBA can produce a good number of roots in cuttings.

After plantlet production under *in vitro* conditions, for minituber production the plantlets were placed in the greenhouse. There are a variety of methods for moving plantlets to greenhouse. Besides using different containers and planting beds time, light intensity and other growth conditions were also considered.

Obradovic and Sukha (1993) showed that planting bed with 80% of vermiculite and 20% of sand was found to be best for plantlet growth that resulted in the production of many minitubers in the greenhouse.

Materials and Methods

In this study Agria and Marfona potato cultivars were planted in pots containing soil and fine sand under greenhouse conditions. When potato plants grew enough, apical buds were removed for growing lateral buds and producing more branches. Then these plants were moved to thermotherapy room and they were kept under 7500 lux light intensity with 16 h photoperiod at 37_C and relative humidity of 60 - 65 %.

The pots were moved to laboratory for meristem isolation after 4 - 5 weeks. Lateral buds were used for meristem isolation under complete aseptic

conditions in the laboratory. Isolated meristems were placed on filter paper bridge in liquid medium. Modified MS medium with GA₃ (0.25 mg/l) and calcium pantothenate (2.0 mg/l) were applied at this stage.

The meristem-derived plantlets were micropropagated in the solid medium by single node cuttings. Special flasks containing 40 ml of modified solid medium (MS) with 0.25 mg/l GA₃ and 0.01 mg/l NAA were used at this stage. Plantlets were grown at 25_C and 16 h photoperiod with 2000 lux light intensity for one month. Then these plantlets were used for this experiment.

The experimental design was factorial laid out in completely randomized design with three factors. In this experiment Agria and Marfona cultivars and each of NAA and BAP were used as factors. Hormones had four levels: 0, 0.5, 1 and 1.5 mg/l. Modified MS solid medium with GA₃ (0.25 mg/l) and calcium pantothenate (2 mg/l) were applied in this experiment. The treatments were replicated six times. Filter sterilized calcium pantothenate was added to the medium inside a laminar flow.

Apical buds and roots of plantlets from previous stage were removed and each plantlet was divided into 4 - 8 cuttings. Three single node cuttings were placed in each flask. Samples were transferred to the growth chamber maintained at 25_C providing 16 hrs photoperiod of 2000 lux light intensity. Then the effects of NAA and BAP were investigated on root number, plantlet length, nodule number, large root length and leaf size and the best hormone combination was selected.

Plantlets of Agria and Marfona were raised using single node cuttings under optimum light intensity and temperature for one month. Then they were moved to greenhouse, and the effects of four planting beds were studied on plantlet growth in the greenhouse. Planting beds were prepared using perlite/sand/loamy soil in the ratio of 1 : 1 : 1, peat moss/sand in 4 : 1 ratio, loamy soil/leaf compost/sand in equal ratio and hull rice/loamy soil in 2 : 1 ratio. Plastic pots of 15 cm diam were used. One plantlet was transferred to each pot. Observations were recorded after one month for growth rate and other characteristics.

Results and Discussion

Significant differences between Agria and Marfona cultivars were observed in respect of all traits (plantlet length, node number and large root length) except leaf size.

Plantlet length (3.62 cm) and node number (5.41) in Agria cultivar were more than Morfona cultivar (3.32 cm and 4.79, respectively).

Root number (3.97) and length of large root (4.06 cm) in Marfona were more than that recorded in Agria cultivar (3.18 and 3.30 cm, respectively).

The effect of using hormone combinations was different on root and shoot formation in plantlets resulting from meristem culture. Application of BAP and NAA decreased shooting and rooting of single nodes. BAP decreased plantlet length and node number in both potato cultivars, but its effect on Marfona cultivar was more pronounced. Treatment without BAP produced taller plantlets (5.8 cm) and more node number (8.35) (Fig. 1). But plantlets with BAP treatment (1.5 mg/l) were short (2.48 cm) and had low number of nodes (3.79). BAP also decreased leaf size in both the cultivars.

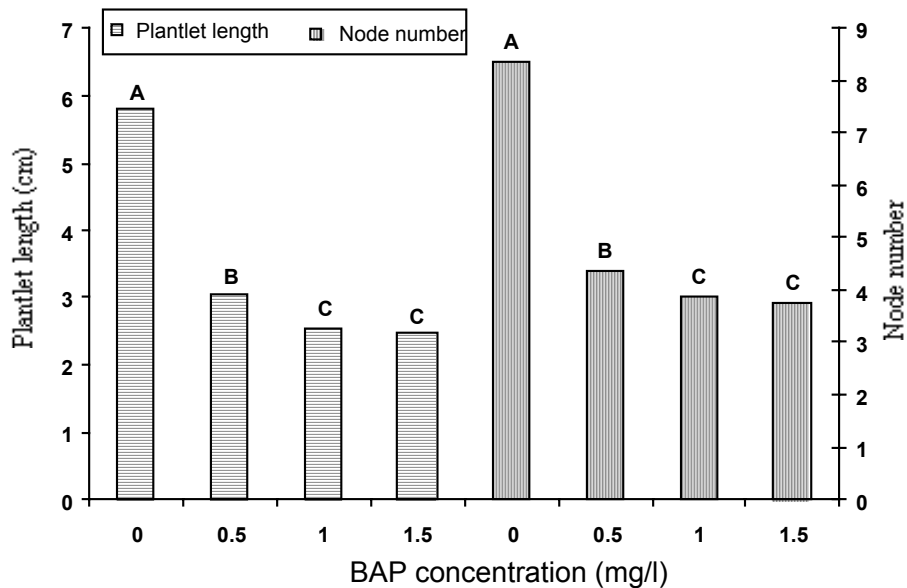


Fig. 1. Effect of different BAP concentrations on plantlet length and node number.

Application of NAA decreased root number and large root length in both Agria and Morfona potato cultivars. This reduction was more in Agria than in Marfona. NAA at 1.5 mg/l reduced the root number (2 - 60) and root length (2.93 cm) (Fig. 2).

Interaction effects between BAP and NAA were significant at 1% level for plantlet length, root number and large root length. Interaction effects between cultivar and hormone (BAP and NAA) were significant at 1% level for

root number and greater root length. Among cultivars, BAP and NAA were significant at 1% level for root number and greater root length.

In greenhouse, planting beds showed different effects on plantlet growth. There were significant differences between peat moss/sand (4 : 1) and other planting beds. The height, stem diameter and leaf size in treated plants with peat moss/sand (4 : 1) were more than other plants. Leaves in treated plants with peat moss/sand (4 : 1) had mostly 5 - 7 leaflets, whereas in other treated plants there were rarely three leaflets.

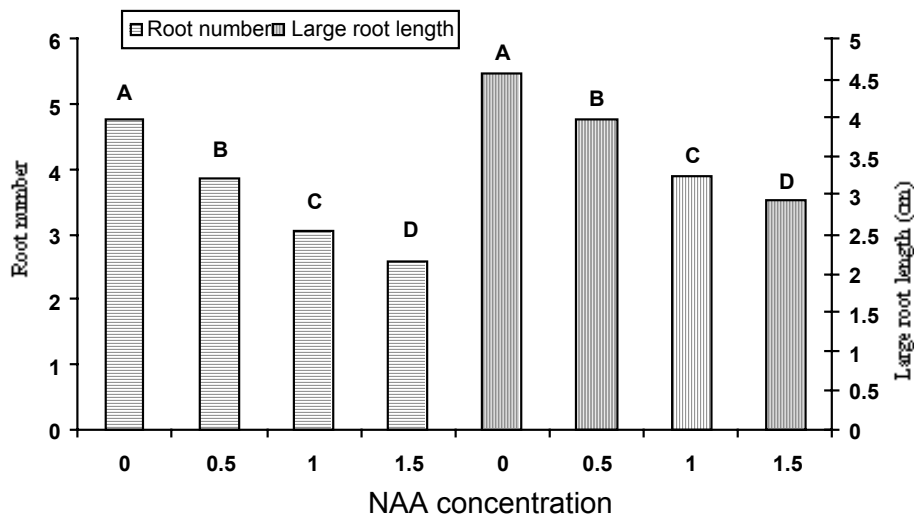


Fig. 2. Effects of different NAA concentrations on number and length of roots.

There were no significant effects among other three planting beds (perlite/sand/loamy soil, hull rice/loamy soil and loamy soil/leaf compost/sand), but the plant treated with perlite/sand/loamy soil had better growth than the remaining treated plants. Marfona had more stem length and diameter than Agria.

The present study showed that lower concentrations of auxin and cytokinin influenced the formation of both root and shoot but at higher concentrations (more than 0.5 mg/l) plantlets showed reversed trends.

In the light of the results obtained in the present study the use of low concentration of hormone is suggested. The planting beds in the greenhouse should contain less or no soil.

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