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Optimisation of Callus Induction in the Leaf and Stem Tissues of the Orange Flesh Sweet potato Variety Bhu Sona

Sweet potato (*Ipomoea batatas* (L.) Lam), belonging to the family Convolvulaceae, is one of the important food cropscultivated globally ~7.4 million hectares, with a total production of ~92 million tons (FAOSTAT, 2019). It is cultivated throughout the tropical and subtropical regions of the world, includingIndia (Edison et al., 2009; Tavva and Nedunchezhiyan, 2012). Because of its shortgrowing period, ability to grow in diverse environmental conditions and high yield potential, sweetpotato is considered as one of the potential cropsthat can help us to meet the future energy and nutritional needs of bothhuman and livestockpopulations particularly in developing countries (Motsa et al., 2015; de Albuquerque et al., 2019). The introduction of β-carotene rich orange-fleshed sweet potato varietiesin dietary programs has alleviatedvitamin-A deficiency among children and pregnant women in many developing countries (Girard et al., 2017; Govender et al., 2019). In India, ICAR-CTCRI and different AICRP-TC centres have released six β-carotene rich varieties *viz*., Bhu Sona, Bhu Kanti, BhuJa, Gouri, Kamala Sundari and Co-5 (Sunitha et al., 2018). Development of in *vitro* plant regeneration from various plant tissues is important for various basic biotechnology applications such as virus elimination, germplasm conservation, including plant propagationand genetic improvement (Arathi et al., 2019; Ravi et al., 2020; Lenka et al., 2018). In tissue culture, plant hormones influence the differentiation and growth of the explants (Hill and Schaller, 2013). In addition to these, several factors, including genetic factors/genotype differences, the type of explants used for regeneration, the selection of hormones and the concentration used, are also known to determine the regeneration efficiency of the plants (Salari et al., 2013).

Several studies have shown that the callus induction and regeneration efficiency in many crop plants, including sweet potato is highly genotype dependent (Ravi and Indira, 1999; Salari et al., 2013; Arathi et al., 2019). Due to these differential responses of the cultivars, genotype specific callus induction protocol needs to be developed for *in vitro* culture based genetic improvement studies (Salari et al., 2013; Arathi et al., 2019). Moreover, development of callus induction protocols would be helpful in the improvement of important traits through biotechnological approaches (Muthusamy et al., 2017; Lenka et al., 2019; Jagannadham et al., 2021). Arathi et al., (2019) used the growth regulators *viz.,* BAP, TDZ, GA_3 and IBA and found that the MS media supplemented with 0.5 mg l^{-1} BAP and 1.0 mg l^{-1} GA_3 displayed good shooting efficiency in sweet potato. Several workers had shown that Naphthalene Acetic Acid (NAA), a synthetic auxin hormone has good effect on callus induction in many crops (Ahmad and Spoor, 1999; Nazir et al., 2020). The effect of NAA on induction of callusing is yet to be studied in the orange-fleshed sweet potato Bhu Sona. Hence, in the present study, the NAA hormone was used for optimisation of callus induction in leaf, stems and root tissues of the Bhu Sona variety.

The study was conducted in the ICAR-Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, during the period 2020-2021. Bhu Sona, sweet potato variety of ICAR-CTCRI, was used in this study to optimise callus induction. Microbial contamination emanating from both endogenous and exogenous sources remains one of the serious concerns in the *in vitro* culture (Amissah et al., 2016). To reduce microbial contamination from the outside environment, first, we initiated the *in vitro* cultures for multiplication of the variety Bhu Sona. The four weeks old plantlets were cut into a smaller size of 3-4 cm shoots and multiplied in the sterile tubes containing MS media with 3% sucrose and 0.8% charcoal. The plantlets were grown in controlled conditions at 25±2°C temperature under a photoperiod of 16 h light and 8-h dark in the plant tissue culture room. Contamination free, four weeks old grown plantlets with uniform size were selected to obtain explants required for callus induction study (Fig. 1).

Fig. 1. Initiation of contamination free fresh *in vitro* plantlets of Bhu Sona.

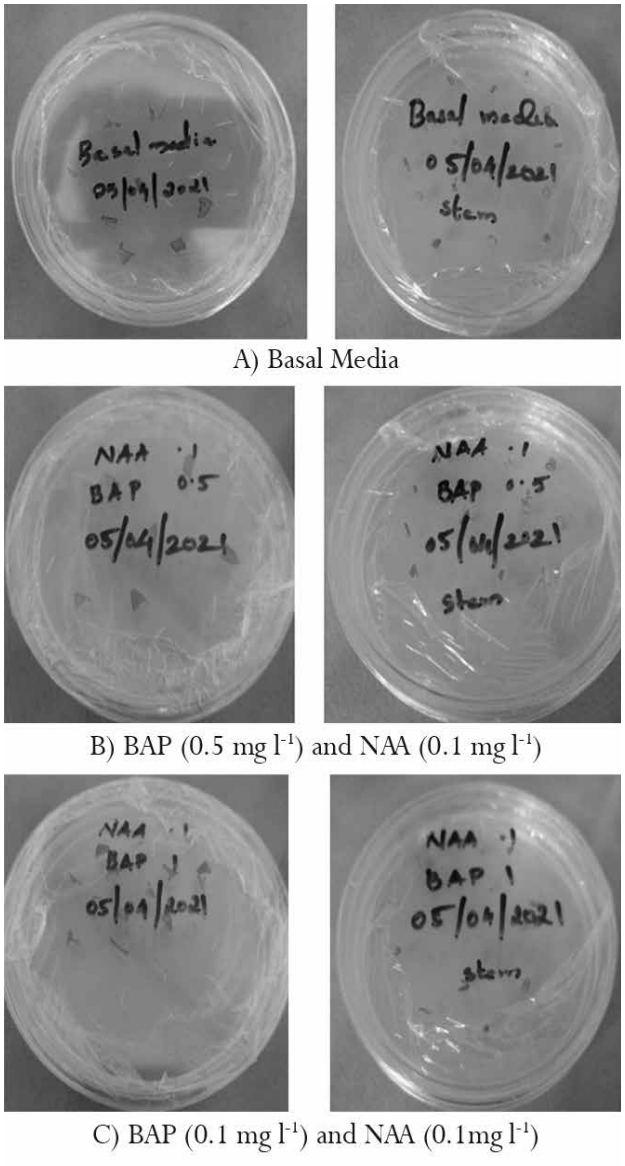


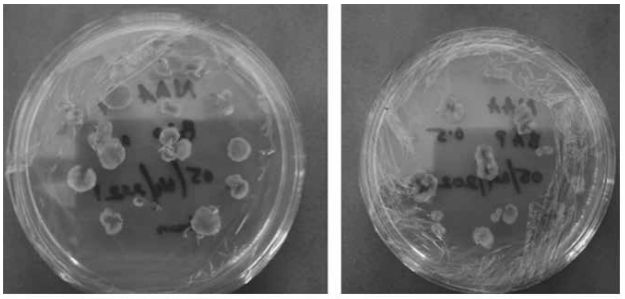
Fig. 2. Culturing of leaf, stem and root tissues of sweet potato for induction of callusing in MS media with different hormone combinations.

The callus induction potential of the variety Bhu Sona was studied in different hormonal combinations *viz.,* Basal media (MS media supplemented with 3% sucrose); basal media supplemented with 0.1 mg l^{-1} NAA+0.1 mg l^{-1} BAP and basal media supplemented with 0.1 mg l^{-1} NAA+0.5 mg l^{-1} BAP (Table. 1 and Fig. 2). Leaf, stem and root segments, about (0.3 to 0.8 cm) long were excised from four weeks-old *in vitro* raised plants and cultured in the petri dishes containing three media combinations *viz.,* Basal media (MS media with 3% sucrose), basal media supplemented with 0.1 mg l^{-1} NAA+0.1 mg l^{-1} BAP and basal media supplemented with 0.1 mg l^{-1} NAA+0.5 mg l^{-1} BAP were used to study the effects of plant growth regulators on callus induction from leaf, stem and root tissue of sweet potato. For evaluating callusing efficiency of the leaf tissues 14, 16, and 14 leaf explants were placed in basal media, 0.1 mg l^{-1} NAA+0.5 mg l^{-1} BAP and 0.1 mg l^{-1} NAA+0.1 mg l^{-1} BAP MS plates, respectively whereas, for evaluating stem tissues 18, 13 and 12 stem explants were placed in basal media, 0.1 mg l^{-1} NAA+0.5 mg l^{-1} BAP and 0.1 mg l^{-1} NAA+0.1 mg l^{-1} BAP MS plates, respectively (Table 1 and Fig. 2) and for evaluating root tissue, 8, 6 and 8 root explants were placed in 0.1 mg l^{-1} NAA 0.5 mg l^{-1} BAP and 0.1 mg l^{-1} NAA+0.1 mg l^{-1} BAP MS plates, respectively (Table 1 and Fig. 2). The petri plates were then sealed with parafilm and cultures were incubated in a culture room at 25±2°C temperature under a dark condition. Additionally, charcoal (0.8%) was included in the growth media to act as an adsorbent of phenolic compounds of the cultures (Arathi et al., 2019).

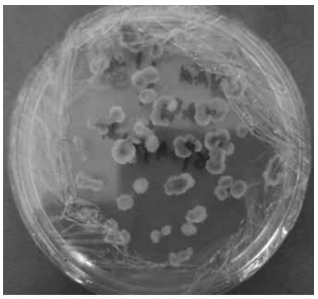
The plates were incubated in dark conditionswith a room temperature of 25±2°C in the tissue culture room. One hundred present callus induction efficiency was observed in both leaf and stem tissues on the MS media supplemented with the hormones NAA and BAP, whereas, in basal media lacking these hormones failed to show the callus induction (Table 1 and Fig. 3). However, the root tissues failed to show callus induction in all three

Table 1. Callus induction in leaf, stem and root tissues of sweet potato under different hormone treatments

Explant	Media Composition	No of Explants inoculated	Callus induced
Leaf	Basal media	14	0
	MS+0.1 mg l^{-1} NAA+0.5 mg l^{-1} BAP	16	16
	MS+0.1 mg l^{-1} NAA+1 mg l^{-1} BAP	14	14
Stem	Basal media	18	0
	MS+0.1 mg l^{-1} NAA+0.5 mg l^{-1} BAP	13	13
	MS+0.1 mg l^{-1} NAA+1 mg l^{-1} BAP	12	12
Root	Basal media	8	0
	MS+0.1 mg l^{-1} NAA+0.5 mg l^{-1} BAP	6	0
	MS+0.1 mg l^{-1} NAA+1 mg l^{-1} BAP	8	0



A) BAP (0.5 mg l^{-1}) and NAA (0.1 mg l^{-1}) (leaf and stem tissues)



B) BAP (0.1 mg l^{-1}) and (0.1mg l^{-1}) (leaf and stem tissues)

Fig. 3. Callusing in leaf, stem and root tissues of sweet potato

media compositions (Table 1 and Fig. 3). A combination of NAA and BAP has shown success in the induction of callusing in many plants (Ahmad and Spoor, 1999; Nazir et al., 2020). Arathi et al. (2019) have used the growth regulators *viz.,* BAPand IBA for induction of the callusing in sweet potato varieties Gowri and Bhu Sona, however, the callus developed from the leaf and stem tissues failed in organogenesis. Hence, in this study we have used the growth regulators *viz.,* BAP and NAA for callus induction. However, thecallus developed from the leaf and stem tissues were failed in organogenesis. Thus, further studies with different hormone combinations, including statistical parameters would be helpful in the standardisation of robust protocol for callus induction and regeneration of the explants in sweet potato.

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