**ABSTRACT**

**Title of the Thesis/Dissertation:**“Comparative study of nano elicitors and their role on growth and development of broccoli (*Brassica oleracea var. italica)* under *in vitro* conditions.”

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| **Degree to be awarded** | **:** | Ph.D. (PlantPhysiology) |
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| **Name of university** | **:** | Sher-e- Kashmir University of Agricultural Sciences &Technology of Jammu. |

The present study “Comparative study of nano elicitors on growth and development of broccoli (*Brassica oleracea var. italica*) under *in vitro* conditions” was carried out at Plant Tissue Culture Laboratory, Division of Plant Physiology, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu during 2020 to 2023 to develop efficient sterilization, callusing, and regeneration protocol under in vitro response using various explants of broccoli. The effect of nanoparticles (40nm) alone or along with other PGRs was studied. There were six treatments viz. Zinc oxide, Copper oxide, silver nitrate, Chitosan, Salicylic acid, and Methyl jasmonate with three different concentrations 10 mg/l,20 mg/l, and 30 mg/l.The result showed that the maximum surface sterilization of leaf explants of Broccoli (*Brassica oleracea var. italica*) was achieved by treatment comprising of 1 % (w/v) bavistin for 5 minutes duration followed by 0.50% (v/v) sodium hypochlorite of 3 minutes and mercuric chloride 0.1% for 0.30 minutes which gave 88.75% uncontaminated cultures and was highest as compared to all other treatments. Maximum callus induction frequency(CIF) (90.47%), was observed on MS media supplemented with 1.5 mg/l 2,4-D and 0.75 mg/l chitosan showed the maximum callus induction frequency (CIF)(90.27%), followed by 87.45% CIF on culture media supplemented with 1.0 mg/l 2,4-D + 0.75 mg/l chitosan. On the other hand, no response was seen on media without growth regulators and nanoparticles. The half-strength MS basal medium, enriched with a precise combination of 2.0 mg/l NAA and 0.5 mg/l IBA, showed best response in root formation (84.25%) and an increased root length of 4.50 cm. Maximum shoot induction (85.25%) and (52.75%) were produced from callus segments maintained on MS medium supplemented with 1.0 mg/l TDZ + 0.25 mg/l IBA and 1.5 mg/l BAP, respectively. the highest average shoot length was recorded at 4.15 cm, followed by 3.49 cm with a combination of 1.0 mg/l TDZ + 0.25 mg/l IBA and 1.5 mg/l BAP. After hardening and acclimatization various morpho-physiological parameters were studied It was found that chitosan consistently recorded the highest relative water content (92.29%).

 Various biochemical parameters viz. total chlorophyll content, total carotenoids, proline, total soluble sugars, glucosinolate content, antioxidant enzyme activities (catalase, peroxidase, superoxide dismutase) nitrate reductase activity, and pollen viability. Nanoparticle application under in vitro conditions reduced and maintained the higher levels of proline, total soluble sugar, total soluble protein, and total free amino acid content. Similarly, the general activity of antioxidant enzymes i.e. catalase, peroxidase, glutathione reductase, and superoxide dismutase was enhanced by the application of nanoparticles in tissue culture condition and their application also enhanced a higher amount of every above parameter. Salicylic acid, zinc oxide, and methyl jasmonate have a more pronounced stimulatory effect on proline and total soluble sugar found in broccoli. Chitosan recorded significantly highest glucosinolate content (8.17μmol/g FW), total biomass (fresh and dry weight of leaves, stem, plant growth, and salicylic acid was found effective in maintaining higher antioxidant enzyme activity(superoxide dismutase (SOD) (9.76**-**units/mg protein), catalase (12.20 units/mg protein, peroxidase (13.26-units/mg protein.), leaf area 219.02 cm² and also emerged as the most effective in keeping pollen viability(80.01%) and reducing flower shedding (29.01%). Methyl jasmonate exhibited the most significant effect, resulting in increasing curd diameter. With an increase in concentration and duration of nanoparticles, all physio morphological parameters showed a linear reduction. The in vitro-generated plants took fewer days to acclimatize in hydroponics as compared to pots. No abnormal effect of nanoparticles was observed on leaf shape and colour except in silver nitrate-treated in vitro plants their leaves were yellow in colour and plants were stunted, molecular markers (SSR) were used to observe the genetic variation among developed in vitro lines of broccoli under in vitro conditions. Molecular marker study showed that silver nitrate treated in vitro plants were the most distinct from the control plants, rest all other in-vitro plants were almost similar with similarity values of 99 to 100 % respectively. Developed in-vitro methodology from the present investigation may be valuable for commercial micropropagation of broccoli.

**Keywords** *Broccoli, Hydroponic, Nanoparticles, antioxidant enzymes, tissue culture, pigments, SSR markers.*

**Signature of Major Advisor Signature of Student**