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# Optimising micropropagation of brussels sprouts and Chinese cabbage: A biotechnological approach toward sustainable crop production

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**Abstract**
Micropropagation gives a rapid and reliable approach for large scale production of disease-free plants overcoming the limitations of conventional farming. Sustainable agriculture demands new innovative approaches to increase food productivity while protecting the environment for future generations. The study focuses on two nutraceutically and economically important Brassicaceae crops: Brussels sprouts (*Brassica oleracea* var. *gemmifera*) and Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), evaluated for *in vitro* growth in different media (Morishige and Skoog-MS, B5, and White medium) along with the effects of plant growth regulators.
Uniform growth of plantlets was observed throughout the study period. Growth parameters, including shoot length, number of nodes and callus formation, were recorded over 60 days. Results showed that plantlet growth was enhanced with PGRs, and callus formation was observed in medium containing 2,4-D. The synergistic combination of 2 ppm BAP and 1 ppm NAA in MS medium led to the most significant shoot and node proliferation, producing an average of 6 shoots in Brussels sprouts and 5 nodes in Chinese cabbage by Day 60.
Commercial micropropagation of these crops makes large scale cultivation of crops a possibility, which promotes sustainability. agriculture and the accessibility to nutraceutical-rich vegetables that have been shown to have anticancer and immunomodulatory properties.

**Keywords:** Brassicaceae, brussels sprouts, Chinese cabbage, micropropagation, sustainable agriculture

## Introduction
Agriculture is the practice of cultivating crops for both food and non-food products. It plays important role in economic progress of developing countries. The world's population is increasing at alarming rate leading to increased food demand. Countries are forced to find alternatives to meet these food demands. This creates an urgent need for developing and implementing different new approaches aiming at maintaining productivity while minimising environmental impact. Sustainable agricultural practices offer solutions for producing food as well as other agricultural products at a lower cost that does not threaten food accessibility and availability, as well as future generations' general well-being [1]. Traditional agricultural practices are commonly affected by seasonality, disease susceptibility, and variability of quality. In order to enhance the crop productivity and to enable sustainable food security, adopting time-tested biotechnological tools has become essential. Micropropagation, though not new, is an established method for sustainable crop production, quick, mass production of disease-free, genetically identical, and high-yielding plantlets under *in vitro* conditions. By using *in vitro* techniques, it overcomes the limitations of traditional farming, particularly in low germination rate or slow growing crops. This method is important for both small scale farmers as well as large agricultural industries ensuring stable yields [2].
*Brassica* is the largest genus among Brassicaceae family consisting of with around 37 species. Vegetables such as broccoli, kale, cauliflower, and Chinese cabbage are rated among the top ten global crops because of their rich nutrients and health promoting phytochemicals. These vegetables show biological activities like antibacterial, anticancer and antiviral activity. They also act as a powerful modulator in the innate immune response system. Frequent consumption is associated to lower risks of cancer, heart disease, immune issues, and other degenerative diseases [3]. Several vegetables are well-known for their nutritional

quality and market appeal; among them Brussels sprouts and Chinese cabbage have gained interest because of their global use and therapeutic benefits [4]. They are also popular for their nutritional value and commercial significance. Use of tissue culture techniques in propagation of these plants allows improved breeding, improved disease resistance, and faster multiplication rates.

Micropropagation has been successfully applied to various *Brassica* species, but standardised and optimised protocols for certain economically important members such as Brussels sprouts and Chinese cabbage have not been done. Prior studies have mostly focused on regeneration in Chinese cabbage from hypocotyl and cotyledon explants [5] but hardly evaluated effect of different basal media and plant growth regulator (PGR) combinations for these two species. Studies in other Brassica crops such as kale, show importance of explant, medium and hormone interactions [6]. Variations in shoot proliferation under different culture conditions are not yet well-documented. Our study therefore is novel in systematically evaluating multiple culture media (MS, B5, White) and varying concentrations of auxins and cytokinin's (BAP, NAA, IAA, KIN, 2,4-D) specifically for Brussels sprouts and Chinese cabbage.

This study highlights species-specific responses and optimising shoot proliferation by filling existing knowledge gaps and contributing to the development of efficient, reproducible micropropagation systems required for large-scale, disease-free, and uniform plant production, thereby supporting broader goals of sustainable agriculture.

## Materials and Methods

### Plant Material

Seeds of Chinese cabbage and Brussels sprouts were collected from a certified commercial supplier.

### Surface Sterilisation of sample

The seeds were surface sterilised using a multi-step procedure to remove debris and eliminate microbial contamination.

### Inoculation and subculture

The sterilised seeds were inoculated on MS medium and initial growth was observed. After seedlings developed, explants were excised and sub-cultured under aseptic conditions on different culture media (MS, B5 & White) containing different concentrations of plant growth regulators (PGRs) to evaluate their influence on growth parameters. The cultures were maintained at a temperature of 22 ± 2 °C under a 16-hour photoperiod using white light. Experiments were conducted in triplicates, with each treatment consisting of multiple independent culture vessels to ensure reproducibility and reliability of the results.

### Plant Medium for *in vitro* growth of plantlets

*In vitro* culture was done on three different media namely Morishige and Skoog (MS) Medium, enriched with macronutrients, micronutrients, vitamins, and a carbon source; B5 Medium, a standard culture medium used for callus formation; and White Medium, primarily used for root growth and germination. Each medium was supplemented with varying concentrations (1, 2, and 3 ppm) of PGR namely IAA, NAA, 2,4-D, KIN, and BAP. The pH of all media was adjusted to acidic (5.7) followed by autoclaving at 121°C for 20 minutes. Sucrose (3%) was added as a carbon source, and 0.7-0.8% agar was included as a solidifying agent.

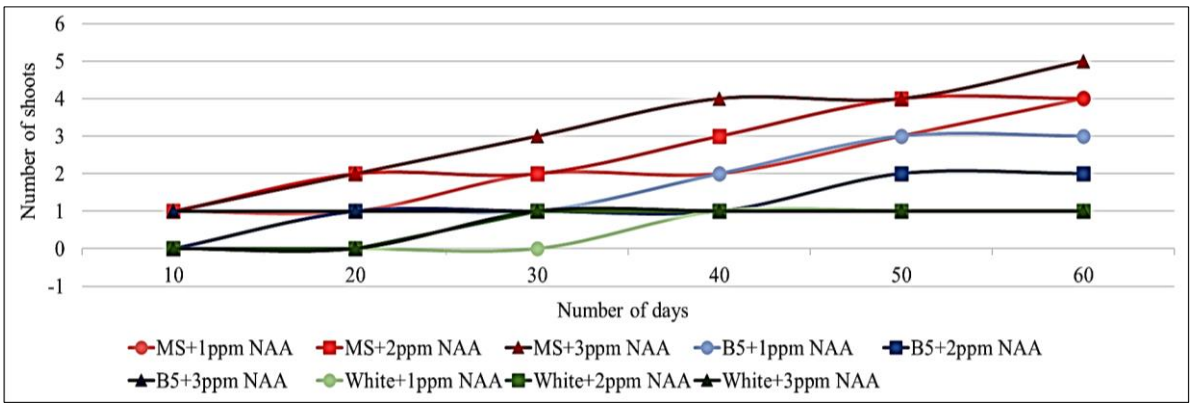
Based on preliminary observations indicating enhanced plantlet growth in MS medium supplemented with 2 ppm BAP and 1 ppm NAA, this specific combination was further investigated to assess its synergistic effect on shoot and node proliferation.

### Data Collection Protocol

Initial seed germination was monitored on MS medium, followed by subculture onto different media with various PGR concentrations. Observations were recorded at 10-day intervals over a 60-day period. Key growth parameters assessed included the number of nodes in Chinese cabbage, the number of shoots in Brussels sprouts, and the extent of callus induction.

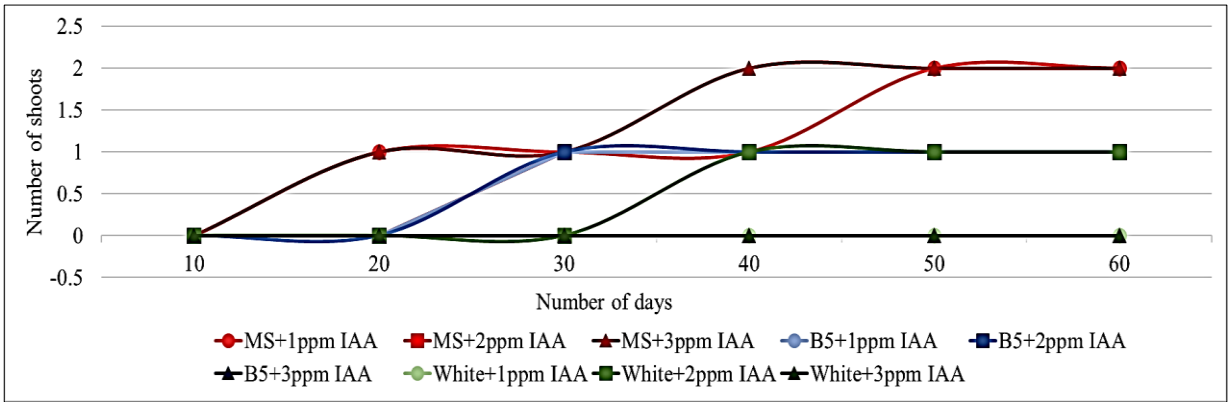
## Results

Successful germination of both Chinese cabbage and Brussels sprouts seeds was observed within 7-10 days on MS basal medium. The germinated seedlings were healthy, uniform, and suitable for further subculture in different culture media (MS, B5, and White) and varying concentrations of plant growth regulators (1, 2, and 3 ppm of IAA, NAA, 2,4-D, KIN, and BAP). Growth responses were monitored at 10-day intervals over a 60-day period. MS, B5 and White media with 1, 2 and 3ppm of IAA, NAA, BAP, KIN showed steady growth of plantlets. In contrast, media containing 2,4-D showed reduced nodal growth, which aligns with its known role in promoting callus induction over organogenesis. The callus was friable, cream to pale yellow in colour, and primarily developed at the base of explants.



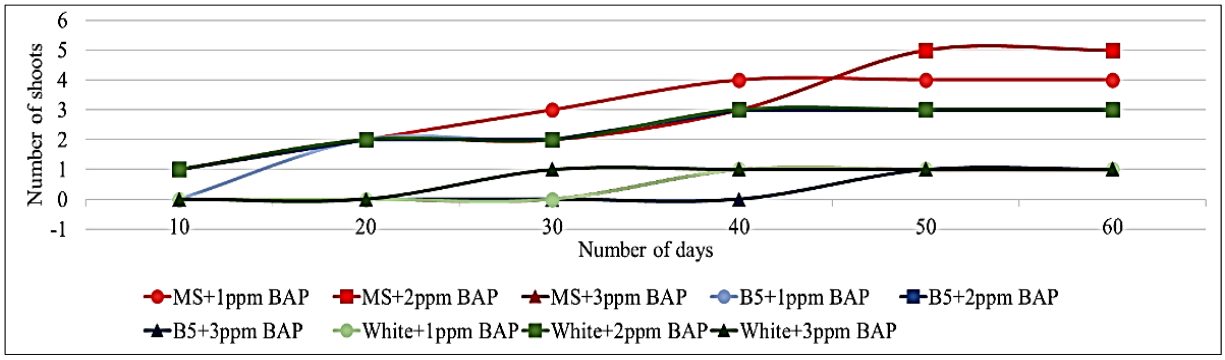

**Graph 1:** Number of shoots of Brussels sprouts in MS, B5 and White Media with 1,2 and 3 ppm NAA

Graph 1 shows steady rise in shoot number observed in MS medium with increasing NAA concentration, reaching a maximum of 5 shoots at 3 ppm by day 60. B5 medium showed moderate response with 3 shoots at lower concentrations, while White medium remained weak throughout. Hence, MS + 3 ppm NAA proved most effective for shoot initiation and growth.



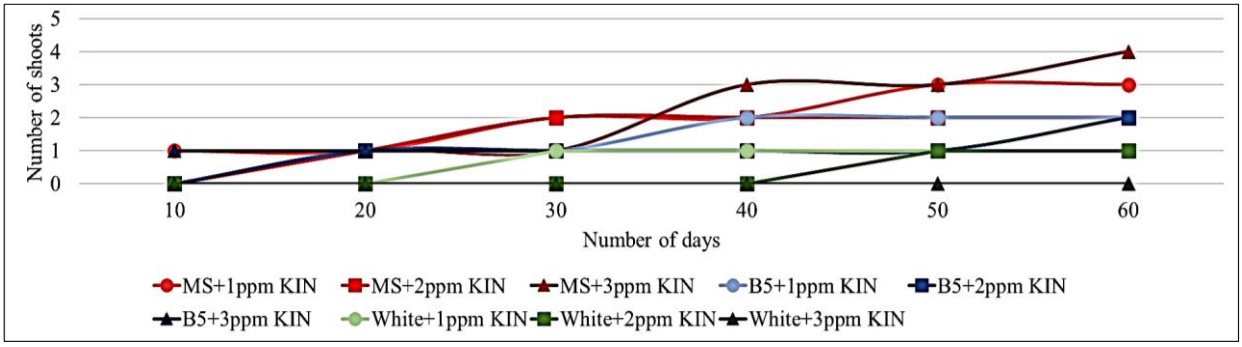

**Graph 2:** Number of shoots of Brussels sprouts in MS, B5 and White Media with 1,2 and 3 ppm IAA

Graph 2 shows MS medium with 3 ppm IAA supported slight shoot development up to 2 shoots by day 60, while B5 and White media showed minimal response.



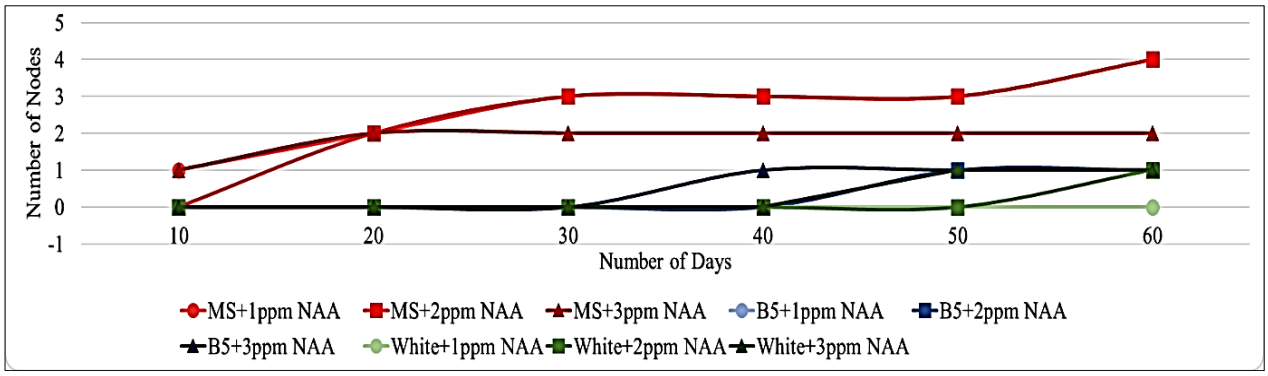

**Graph 3:** Number of shoots of Brussels sprouts in MS, B5 and White Media with 1,2 and 3 ppm BAP

Graph 3 shows the combination of MS medium with 2 ppm BAP produced the highest number of shoots (up to 5) by day 60, while 3 ppm was less effective. B5 and White media. showed limited response even at higher concentrations.



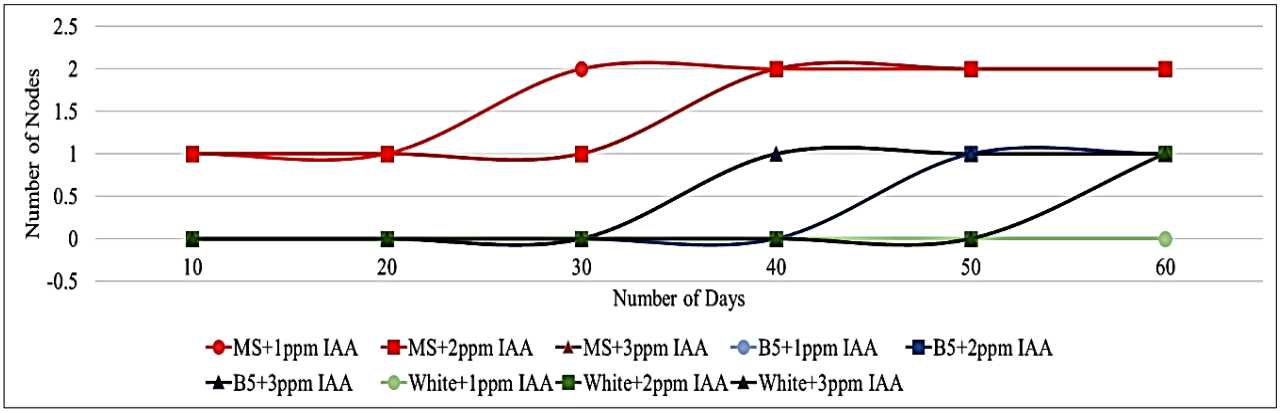

**Graph 4:** Number of shoots of Brussels sprouts in MS, B5 and White Media with 1,2 and 3 ppm KIN

Graph 4 shows MS medium with 3 ppm KIN produced a maximum of 4 shoots by day 60. B5 and White media showed minimal response.



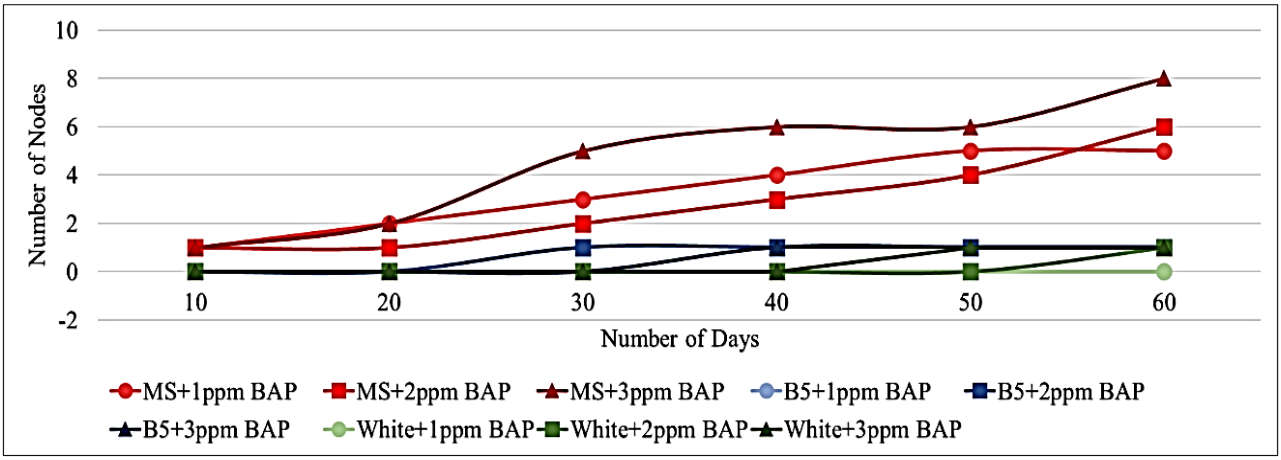

**Graph 5:** Number of nodes of Chinese Cabbage in MS, B5 and White Media with 1,2 and 3 ppm NAA

Graph 5 shows a gradual increase in shoot number with 1-2 ppm NAA in MS medium, reaching a maximum of 4 shoots by day 60, while higher concentration (3 ppm) slightly reduced response. B5 and White media showed minimal or delayed growth.



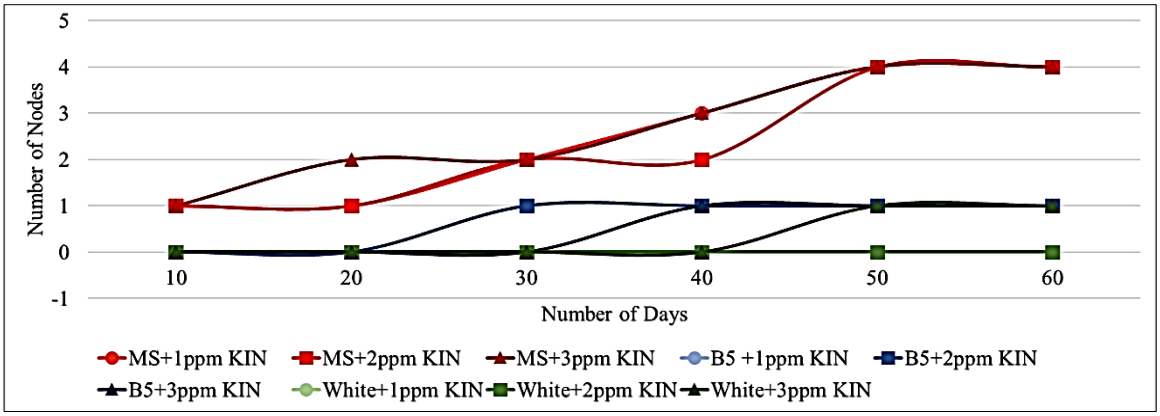

**Graph 6:** Number of nodes of Chinese Cabbage in MS, B5 and White Media with 1,2 and 3 ppm IAA

Graph 6 shows shoot initiation under IAA was slower and less pronounced than with NAA. MS medium with 1-2 ppm IAA showed up to 2 shoots by day 60, while other media remained mostly unresponsive.




**Graph 7:** Number of nodes of Chinese Cabbage in MS, B5 and White Media with 1,2 and 3 ppm BAP

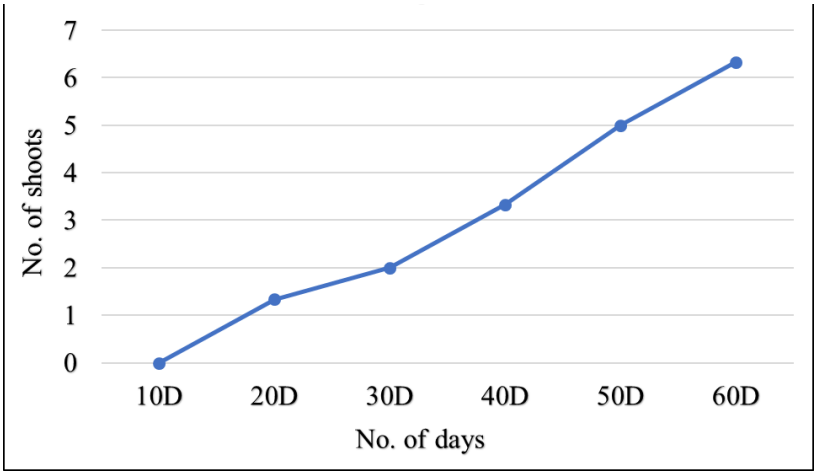
Graph 7 shows positive response in MS medium supplemented with BAP, especially at 3 ppm concentration, which produced up to 8 shoots by day 60. B5 and White media showed poor or delayed response.




**Graph 8:** Number of nodes of Chinese Cabbage in MS, B5 and White Media with 1,2 and 3 ppm KIN

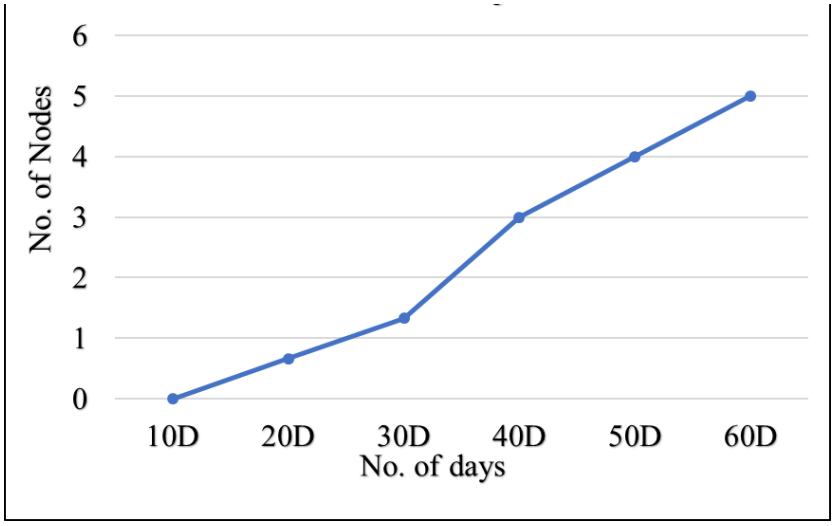
Graph 8 shows MS medium with 3 ppm KIN showed moderate shoot proliferation, producing 4 shoots by day 60, while lower concentrations showed slower growth. B5 and White media exhibited negligible response.

The experiment results revealed that MS medium supplemented with 2 ppm BAP and 1ppm NAA significantly enhanced shoot proliferation in both species. The synergistic effect of the same was studied.



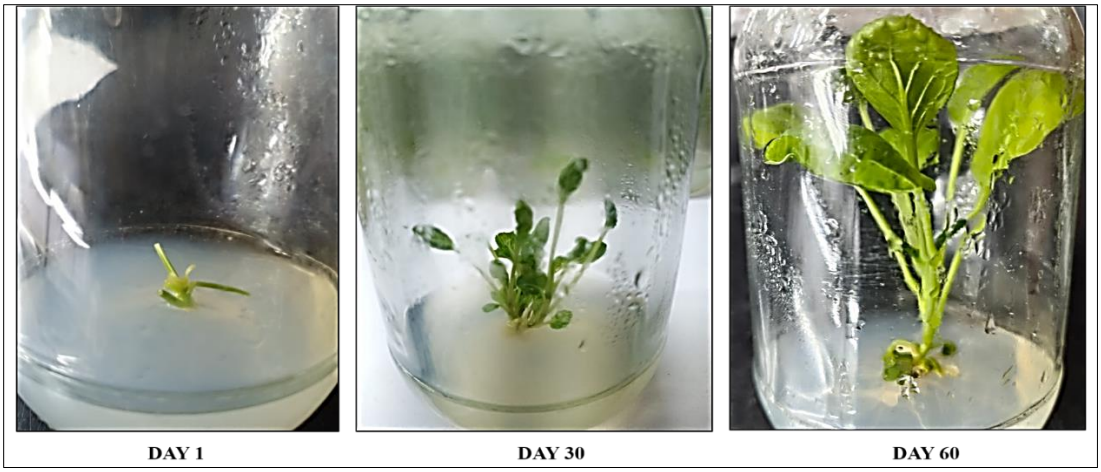

**Graph 9:** Average Number of Shoots grown in MS+2ppm BAP+1ppm NAA of Brussels sprouts

Graph 9 shows average number of shoots increased steadily from day 10 to day 60, showing a clear and continuous rise in growth. The mean shoot count progressed from 1.33 at 20 days to 6.33 at 60 days, indicating active organogenesis and shoot multiplication as the culture period advanced.

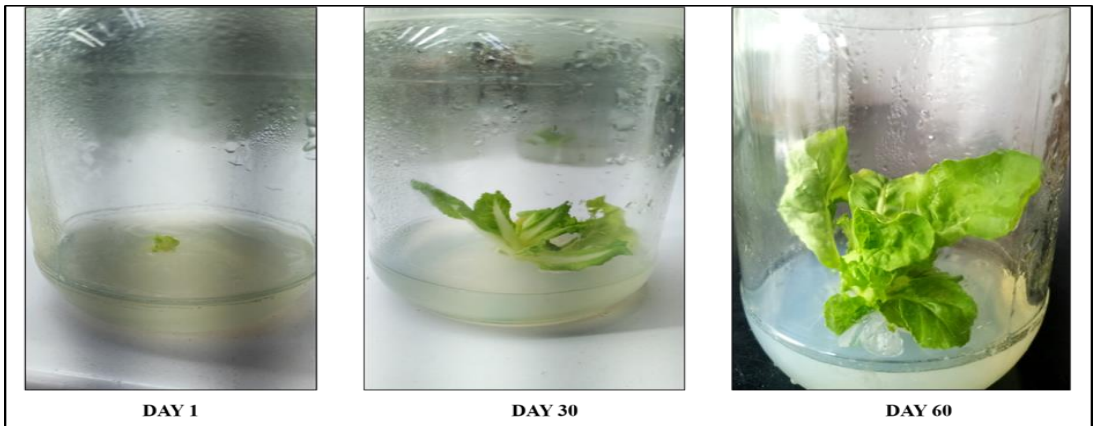



**Graph 10:** Average Number of Shoots grown in MS+2ppm BAP+1ppm NAA of Chinese Cabbage

Graph 10 shows the average shoot number increased gradually with time, starting from 0 at day 10 and reaching 5 by day 60. The steady rise from 0.66 at 20 days to 5 at 60 days reflects continuous shoot initiation and multiplication throughout the culture period, indicating healthy *in vitro* growth and development.




**Photoplate 1:** *In vitro* growth of Brussels sprouts plantlets in MS media with 2ppm BAP and 1ppm NAA




**Photoplate 2:** *In vitro* growth of Chinese Cabbage plantlets in MS media with 2ppm BAP and 1ppm NAA

## Discussion

The present study provides a comprehensive evaluation of plant tissue culture conditions influencing the *in vitro* growth of Brussels sprouts and Chinese cabbage. The results indicated MS medium favoured higher germination rates for both the plants. The more germination rate in Morishige and Skoog medium over B5 and White medium can be attributed to its enriched macronutrient and micronutrient composition, mainly nitrate and ammonium ions that promote rapid cell division and morphogenesis.

Maximum shoot proliferation of Brussels sprouts was seen in MS medium, particularly with 2 ppm and 3 ppm BAP, reaching up to 5 shoots by Day 60. For Chinese Cabbage, the highest node formation occurred in MS medium supplemented with 2 ppm BAP, peaking at 8 nodes by Day 60. B5 medium supported moderate growth, while White medium consistently showed the least response. Higher PGR concentrations (3 ppm) showed limited or plateaued growth, indicating a possible inhibitory or vitrifying effect (as shown in graph 3 and graph 7).

Use of Auxin treatment revealed NAA is more effective than IAA in *in vitro* culture systems due to its greater chemical stability and long-lasting presence in the culture medium. IAA occurs naturally and is very unstable and breaks down quickly when exposed to light or high temperatures. It mostly oxidises within a few hours after preparing the medium. On the other hand, NAA is a synthetic auxin that resists both enzymatic breakdown and photodecomposition. This allows for a more consistent and longer-lasting auxin effect during the culture period. This stability helps NAA provide steady stimulation of cell division and callus initiation [7, 8].

Among the cytokinin's, BAP performed better than KIN in shoot proliferation mainly because of its higher activity and stability in plant tissue culture systems. BAP binds more effectively to cytokinin receptors in meristematic cells, which leads to better stimulation of cell division and shoot initiation. It is also more chemically stable and easier for plant tissues to absorb compared to kinetin. Kinetin tends to break down quickly and shows slower responses. As a result, BAP encourages faster and more synchronised activation of shoot meristems, leading to more shoot growth and elongation [9, 10].

The synergism between auxins (IAA, NAA, 2,4-D) for root and callus formation, and cytokinin's (BAP, KIN) for shoot development, is key to successful micropropagation in Brussels sprouts and Chinese cabbage. Precise regulation of PGR levels according to each plant's developmental stage is crucial for optimal tissue culture outcomes.

The experiment results confirmed that MS medium supplemented with 2 ppm BAP and 1 ppm NAA significantly enhanced shoot proliferation in both species. B5 medium showed a moderate growth, but White medium had a poor response. The varying response to PGRs indicates species-specific needs for optimized micropropagation protocols. The ability to standardize *in vitro* propagation of Brassicaceae vegetables contributes to sustainable agriculture by reducing field losses and enabling year-round production of nutrient-rich crops.

## Conclusion

The study of micropropagation in plants Brussels sprouts and Chinese cabbage showed that using Morishige and Skoog (MS) medium successfully optimised *in vitro* conditions for shoot proliferation and callus formation. B5 and White medium consistently recorded low responses across all treatments, indicating limitations for micropropagation in Brassicaceae crops. The use of MS medium enriched with 2 ppm BAP and 1 ppm NAA proves highly effective for shoot and node formation in both plants, while higher concentrations (3 ppm) led to suppressed or plateaued growth. These findings contribute to the development of refined protocols for *in vitro* propagation of Brassica crops.

Future research can be explored for molecular mechanisms influencing tissue differentiation and genetic stability during micropropagation. Acclimatisation protocols for *ex vitro* transfer should be developed to enhance survival rates and the overall success of micropropagation in these species. The standardised micropropagation protocols developed here can be applied for large-scale commercial cultivation, ensuring uniform, disease-free plantlets. This contributes to sustainable agriculture by enabling year-round production of nutritionally rich Brassicaceae vegetables and reducing losses due to seed variability, pathogens, and seasonal limitations.

## Abbreviations

1. MS - Murashige and Skoog Medium
2. B5 - Gamborg's B5 Medium
3. PGR - Plant growth regulators
4. IAA - Indole-3-acetic Acid
5. NAA - 1-Naphthaleneacetic Acid
6. 2,4D - 2,4-Dichlorophenoxyacetic Acid
7. BAP - 6-Benzylaminopurine
8. KIN - Kinetin

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