Research Article

Cost Effective and Season Independent Micropropagation Protocol for *Ficus carica* L. using Plant Natural Growth Substances (PNGS)

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Abstract | *Ficus carica* (Fig) is a native Asian tree with great nutritional, cultural, and environmental significance. The consequences of climate change and shortening growing seasons necessitate for a cost-effective, season independent, simple and potent propagation method. *In vitro* propagation has enormous potential but high cost of commercial substances is the primary barrier in dissemination of technology to end users. Plant Natural Growth Substances (PNGS) are cheap and locally available alternatives that can reduce the cost of the technology. In the present study, fig cuttings were treated with both the commercial substances and for 48 hours followed by transplantation into the soil. Murashige and Skoog media (1/10 X) supplemented with respective growth hormones/regulators was used as watering medium. All of the PNGS performed at par with their commercial counterparts. PNGS-1 and PNGS-4 showed comparatively better responses than both the standards and control. PNGS supplemented media produced root primordia earlier which in turn promoted earlier root induction and higher survival rates. Furthermore, PNGS-1 and PNGS-4 increased root lengths and an increase in number of roots was recorded under media containing PNGS-1, 2, and 4. Overall PNGS-4 showed the most significant results followed by PNGS-1. In conclusion, PNGS extracts outperformed the commercial counterparts and exhibited highly significant results. PNGS can safely be described as highly potent and cost-effective alternatives to commercially available growth hormone and regulators. They enhance the applicability and affordability of *in vitro*-propagation for fig and potentially other woody plants.

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Introduction

Fig is a native Asian fruit tree which stands out for its multifaceted richness intertwining with different aspects of human life and environment. Fig possesses highly significant place in nutrition, culinary practices, history, culture, and the environment of the area (Badgujar *et al.*, 2014; Zhang *et al.*, 2024). Nutritionally, dietary fiber, vitamins, and minerals are profuse constituents of fig fruits (Barolo *et al.*, 2014; Crisosto *et al.*, 2011) while economically, it is cultivated to meet the indigenous consumption needs

as well as, for exporting (Mawa *et al*., 2013; Salma *et al*., 2020). The long roots of the plant prevent soil erosion and improve soil fertility. Fig is home to numerous birds and insects (Mawa *et al*., 2013) and has a symbiotic relationship with wasp (Eisikowitch *et al*., 2022; Galil and Eisikowitch, 1971).

Fig propagation is crucial for farmers to create new trees, preserve desired characteristics, and increase total yield. There are several fig propagation techniques, each with benefits and uses of its own (Hepaksoy and Aksoy, 2006). Fig Propagation is generally carried out for establishing larger orchards stock for commercial fig production, preservation of specific genetic traits in fig plants, developing and introducing new fig cultivars to increase yield, enhance resistance to pests and diseases and adaptation to local conditions (Shamsuddin *et al*., 2021). Development of an effective propagation protocol will enhance growth and productivity of a region by promoting effective crop management, conservation programs and sustainable agriculture. In conclusion, fig propagation is essential to the growth and success of fig agriculture going forward. It promotes genetic variety, permits plant growth, facilitates the creation of cultivars, and guarantees effective and sustainable fig production (Mafrica *et al*., 2025).

Figs are susceptible to certain diseases and pests and when effected can instigate the spread of infection in propagated plant material (Aljane *et al*., 2018). Long juvenile stage and high genetic variability of the fig plant is also a hinderance in propagation through seed. It not only takes 6-7 years to produce seeds but genetic variability results in the loss of desired traits (Mars, 2001; Muhammad *et al*., 2021). Another challenge is the environmental factors which are worsened by the climatic changes as the normal growth periods are reduced and seasons are shifting. The reproducibility of the available propagation protocols is not up to the mark and show difficulties in rooting (Hepaksoy and Aksoy, 2006). Grafting is another procedure that can be used for fig propagation but rootstock compatibility is a challenge. Variations in rootstocks can affect plant growth, disease resistance, and soil-specific adaptability (Bester *et al*., 2023; Saddoud *et al*., 2008).

In vitro propagation is very helpful for propagating figs because it enables quick production of a large number of genetically identical plants from a small amount of plant material (Kumar *et al*., 1998). *In vitro* propagation offers rapid multiplication of plants by producing a large number of genetically identical copies in short time. Due to higher concentration of growth hormones infections and disease are unable to propagate in the cultured stock and disease-free plantlets can be produced. Plant tissue culture is also season independent and can produce plantlets throughout the year along with maintained genetic uniformity and desired traits (Dhage *et al*., 2015; Ling *et al*., 2022). Specialized *in vitro* procedures meet the needs of fig propagation programs but they are economically and mechanically expensive and are not suitable to be used at small and commercial scales.

IBA is a synthetic auxin that has frequently been used in *in vitro* propagative procedures. IBA accelerates the start and growth of roots by promoting cell proliferation and elongation at the base of cuttings (Shinde, 2019). On the other hand, GA_3, a naturally occurring gibberellin, mainly encourages seed germination, stem elongation, and dormancy breaking. Its function in rooting is more complicated, though, low levels of GA_3 can actually initiate the production of roots by breaking dormancy and promoting cellular growth (Ghasemi-Ghehsareh and Khosh-Khui, 2018).

Sriskanda *et al*. (2021) used combination of auxin and cytokinin for the fig propagation. 6-Benzylaminopurine (BAP) promoted the shoot number whereas the combination of BAP and indole-3-acetic acid (IAA) produced the highest shoot length. On the other hand, highest rooting percentage was seen for indole-3-butyric acid (IBA; 83.33%). In another study, MS medium with IBA, gibberellic acid (GA_3) and 6-benzyladenine (BA), was reported to be the best combination for multiplication of fig explants (Hepaksoy and Aksoy, 2006). Ferreira and Pasqual (2005) used gibberellic acid (GA_3) naphthalenacetic acid (NAA) for *in vitro* production of fig plants. The combination of 2 mg. L^{-1} NAA and 8 mg. L^{-1} GA_3 improved root dry matter weight and root elongation which is a huge advantage at the specific protocol stage when rooting is essential.

In conclusion, plant tissue culture is essential to the propagation of figs because it provides benefits such as quick multiplication, genetic homogeneity, disease resistance, and the retention of superior cultivars. This method has developed into a useful

tool in contemporary horticulture, enhancing the productivity and profitability of fig production. Biggest hurdles in disseminating the technology to the consumers is higher cost and specialized conditions of *in vitro* propagation procedures (Chen, 2016). Commercially available growth hormones/ regulators are very expensive and their handling require specialized training and machinery. The cost-productivity ratio is too high for *in vitro* technology and is major hinderance in its dissemination to consumers and small-scale farming practices (Kodym and Zapata-Arias, 2001).

A number of plants extracts have been used for propagation purposes but most of them have been used as nutrients supplements. Only a few reports show the use of natural extracts as growth substances. Natural extracts like aloe vera, seaweed, coconut water, willow bark (Salih *et al*., 2024), garlic (Abbasifar *et al*., 2020), ginger, and papaya have been used in plant propagation. Extracts from ginger and garlic promote roots and have antibacterial properties, which lowers the contamination during the culture process. These natural extracts are not only efficient but also provide a low-cost, sustainable, and eco-friendly substitute for synthetic hormones, particularly in low-input or organic agricultural systems (Gomes *et al*., 2018).

Current results encourage sustainable and accessible fig farming, particularly for small-scale and resource-constrained producers. By eliminating the need for expensive machinery, hormones, or specialized training, this strategy encourages broader adoption, boosts regional output, and encourages agricultural independence in both urban and rural areas.

Materials and Methods

Plant material

Fig apical tips were collected from host plants measuring 6 to 8 cm for explant preparation. The sizes of the explants were standardized to 6 cm to facilitate the comparison of their responses under different growth substances.

Cultures

Cuttings were treated with respective growth substances for 48 hours in a growth room with a 16-hour photoperiod at 27 °C. Then cuttings were transplanted into pots containing sterilized soil, comprised of silt, peat moss, and normal soil in 25:25:50 ratio. Murashige and Skoog (MS) solution (1/10 X) supplemented with the respective growth substance was used to water the pots on a regular basis. Only macro and micronutrients of Murashige and Skoog medium (MS) at one-tenth of the final concentration were used for the medium preparation (Table 1). Iron source and vitamins were not used because they enhance contamination percentages in growth mediums. Following Table 1 shows the composition of watering medium.

Table 1: *Composition of 1/10 MS medium.*

Contents	Concentration
MS Macronutrients	1/10 X
MS Micronutrients	1/10 X
Growth substances	As per standard protocols
*X represents the working concentration of MS Medium	

Growth regulators and plant natural growth substances

Various growth substances were utilized in the study. Two commercially available growth substances (IBA and GA_3) were used as standards. IBA was used at a concentration of 1.5 mg/L (Fráguas *et al*., 2004) while GA_3 was used at a concentration of 0.5 mg/L (Comlekcioglu *et al*., 2007). Four PNGS extracts, PNGS-1, PNGS-2, PNGS-3, and PNGS-4 were used at a concentration of 9 ml/L, equivalent to 0.9 g fresh weight of source plant (Shah and Fahim, 2018). These were prepared from four different plants (Table 2). Online resource 1 (Figure 1) shows pictures of the plants. Arial Parts of the plants were collected and extracted in methanol-water (MW) solution (80:20). One gram of plant material was extracted in 5 ml of MW extraction buffer. Aqueous phase was separated by centrifugation at 5000 rpm for 5 minutes. The pellet was re-extracted in 5 ml MW and both the supernatants were pooled off.

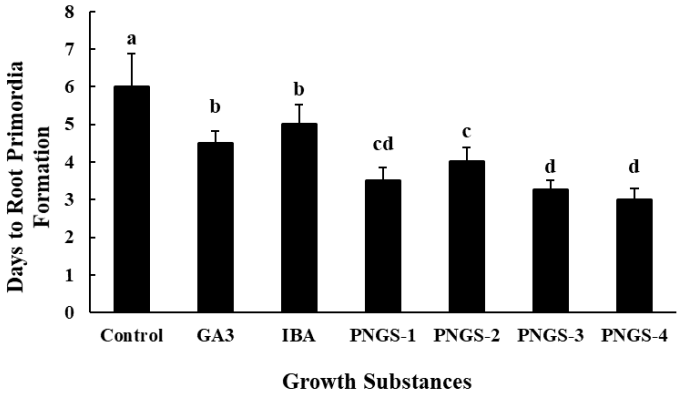

Figure 1: *Effect of PNGS on days to root induction of fig cuttings. n=11 ±SE. Alphabetic labels represent LSD grouping.*

Table 2: *Plants used to prepare plant natural growth substances (PNGS).*

S. No.	Plant	Scientific name	NCBI (txid)	Extract
1	Miracle Leaf	*Kalanchoe pinnata*	80913	PNGS-1
2	Barrel Cactus	*Ferocactus echidne*	867047	PNGS-2
3	Easter Lily Cactus	*Echinopsis oxygona*	----	PNGS-3
4	Monstrose Apple Cactus	*Cereus peruvianus monstrose*	----	PNGS-4

*txid; Taxonomy ID

Growth and transplantation

Ten pots were used for each treatment and three cuttings were planted in each pot. Respective solutions were used to water the cuttings. The experiment was carried simultaneously across three independent replications (10 pots each) to ensure reliable data collection regarding rooting parameters. The cuttings were harvested from three pots every two days and the data was collected. Cuttings were transferred to soil after sufficient development of root system and their survival rates were recorded.

Data recording and analysis

The data regarding days to root primordia formation, days to root induction, number of roots and root lengths was recorded. Days to root primordia formation (DRPF) and days to root induction (DRI) were measured by monitoring explants every two days up to 15th day of cultures. Maximum root length and average root length in cm were noted on 30th day of cultures. Root number was also counted on 30th day of culture initiation. On the other hand, survival rate was noted after transplantation of rooted plantlets in soil. The cuttings were observed up to eight weeks and percentage of survived plantlets was calculated. The data was entered in Microsoft Excel for ANOVA analysis. LSD test was run in Statistix 8.1© while Principal component analysis was carried out in Origin Pro®.

Results

Root primordia formation

Fig explants on PNGS supplemented media induced root primordia significantly earlier than both the standards and control (Figure 1). PNGS-4 was the quickest (3 days) to induce root primordia, followed by PNGS-3 (3.25 days). PNGS-1 took 3.5 days while PNGS-2 induced root primordia in 4 days. GA_3 and IBA were lagging behind with 4.5 and 5 days respectively, while control was the last to induce the primordia (6 days). PNGS-4 and 3 were best performing extracts in terms of root primordia formation.

Root induction

Earlier root primordia formation led to earlier root induction and it was noted that PNGS-4 was the first to induce the roots in 8.15 days followed by PNGS-3 and PNGS-1 with 8.16 and 8.34 days, respectively. On the other hand, GA_3 and IBA induced root in 11.18 and 11 days while control took longest long time to induce roots i.e., 14.69 days (Figure 2). The results indicated that PNGS-4 is more effective than other treatments.

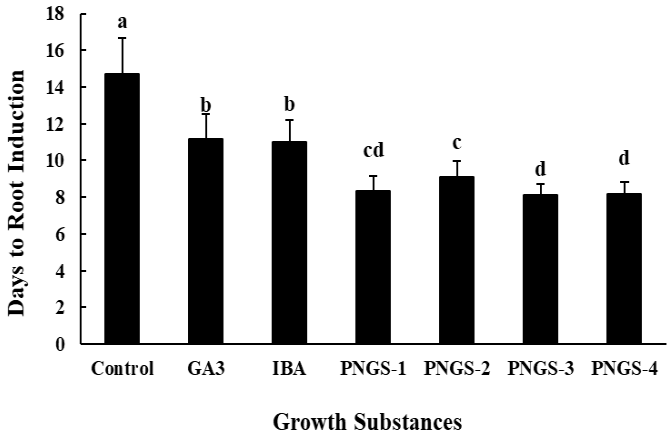

Figure 2: *Effect of PNGS on days to root induction of fig cuttings. n=11 ±SE. Alphabetic labels represent LSD grouping.*

Number of roots

Number of roots were noted on 30th day of culture and the results showed that the application of IBA to the plantlets increased number of roots (8.27 roots) in comparison to GA_3 and the control (7.36 and 6.90 roots per plant, respectively). While, PNGS-2 supplemented growth media produced highest number i.e., 13.31 roots per plant, followed by PNGS-1 with 11.45 roots and PNGS-4 with 10.90 roots. With an average of 7.72 roots per explant, PNGS-3 was seen to be lagging behind in terms of root count (Figure 3). It was observed that PNGS-2 is highly potent growth substance and produces more roots.

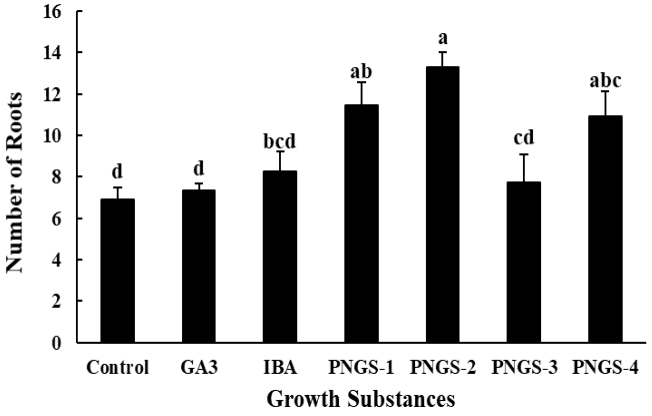

Figure 3: *Effect of PNGS on number of roots of fig cuttings. n=11 ±SE. Alphabetic labels represent LSD grouping.*

Root length

The maximum and the average root lengths of explants under different growth substances were measured on 30th day of culture initiation. PNGS-4 produced the longest roots, with a maximum root length of 12.24, followed by PNGS-1, 3 and 2 with 8.91, 7.25 and 6.74 cm, respectively. Maximum root length in explants under IBA was 4.47 cm while GA_3 showed maximum length of 4.17 cm. Similar pattern was seen for average root lengths where PNGS-4 produced longest roots i.e., 8.69 cm followed by PNGS-3, 2 and 1 with 4.9, 4.8 and 4.1 cm, respectively. IBA showed similar average length to that of PNGS-1 i.e., 4.13 cm while GA_3 and control also showed similar lengths (2.81 and 2.91 cm, respectively; Figure 4). The results demonstrated that PNGS-4 significantly enhances the root lengths as compared to IBA, GA_3, PNGS-1, 2 and 3.

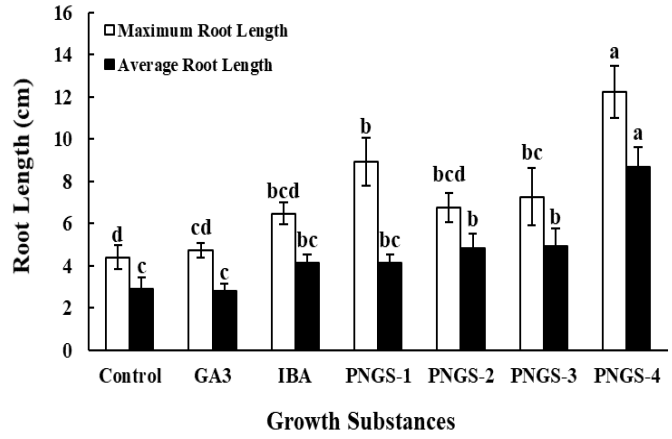

Figure 4: *Effect of PNGS on root lengths of fig cuttings. n=11 ±SE. Alphabetic labels represent LSD grouping.*

Survival percentage

Cultured explants were transplanted into the soil and it was observed that explants on PNGS survived substantially better than standards and control. PNGS-3 performed remarkably better in terms of survival rate in comparison to standards i.e., 83% survival. PNGS-1 and PNGS-4 also exhibited better survival percentages, both with 72%. Explants on PNGS-2 had lowest survival rate i.e., 61 %. IBA showed a survival rate of 50% while under GA_3 44 % survival was recorded. Control showed lowest survival percentage among all the substances i.e., 39% (Figure 5). PNGS extracts enhance the survival percentages of the transplanted cuttings specifically, PNGS-3.

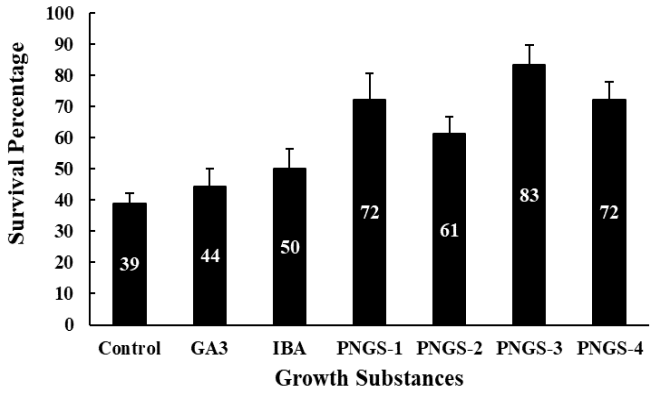

Figure 5: *Effect of PNGS on survival rate of fig cuttings. n=11 for ±SE. Alphabetic labels represent LSD grouping.*

Principal component analysis showed a strong positive correlation between number of roots, root lengths, survival percentages and PNGS-4. Days to root primordia formation and days to root induction were negatively correlated, as PNGS-4 induced the primordia and roots earlier than other substances (Figure 6). This correlation was seen across both principal components and accounted for a cumulative variance percentage of 87. All the PNGS showed similar patterns on Principal Component 1 (PC1; 75.87 % variance) having strong correlations with average and maximum root length, number of roots and survival rates. However, PC2 (11% variance) showed that PNGS-3 has negative correlation with morphological parameters. On the other hand, days to root primordia formation and days to root induction showed strong negative correlation with all the PNGS extracts on PC1.

Discussion

Success of a plant propagation program is dependent on efficacy, applicability and cost-effectiveness of the program. Efficacy can be determined by measuring the morphological growth parameters of the cultured plantlets, specifically those related to rooting, because in organized cultures shoot system is already present

and only rooting is required (Grossnickle, 2012). These parameters include formation of root primordia, root induction, root lengths and root number. The survival rate of the plantlets is the primary objective of any propagation program which is principally dependent on these rooting parameters (Georget *et al.*, 2017).

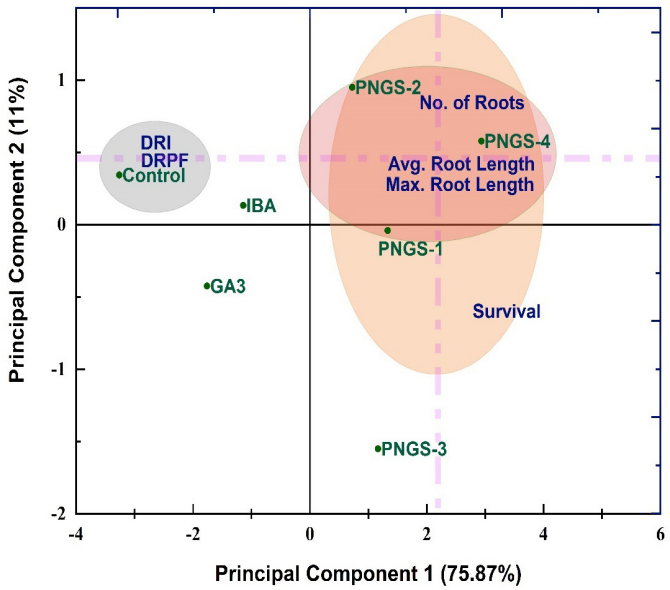

Figure 6: *Principal component analysis of morphological parameter of fig explants against growth substances in in vitro cultures (DRPF: days to root primordia formation and DRI: days to root induction).*

A crucial factor in determining the plant's capacity to adjust under changing soil conditions is formation of new root primordia followed by induction of roots. Better the root system healthier and vigorous is the plant and higher the productivity (Koevoets *et al.*, 2016). Fig explants on PNGS extracts induced root primordia significantly earlier than both the standards and control. GA_3 and IBA took longer times than PNGS while control was the last to induce primordia (Figure 1). Similar pattern was recorded for root primordia formation where explants under control conditions took long time to induce roots, while addition of GA_3 and IBA to growth medium reduced the time for root induction, significantly. Supplementation of PNGS extracts to medium further reduced the time for root induction by 35%, in comparison with GA_3 and IBA (Figure 2). The results reveal that PNGS possess rooting hormones which promote the formation of root primordia and root induction earlier than other substances. Our results also indicate that PNGS extracts not only possess the rooting hormones but also have a balanced composition of other growth promoting substances which results in promotion of rooting. Environmental conditions influence the growth of roots, influencing decisions about where and when to produce new lateral roots as well as how quickly and in which direction to grow (Malamy, 2005). The chemical components of the environment that influence the primordia formation and root induction are growth substances. The key to succeeding in the culture is selecting the right kind and combination of growth substances (Brunoni *et al.*, 2022) in appropriate relative concentrations (Rademacher, 2000; Saini *et al.*, 2013). Among these hormones GA_3 is an initiator of any growth pathway, while auxins promote root induction (Saini *et al.*, 2013).

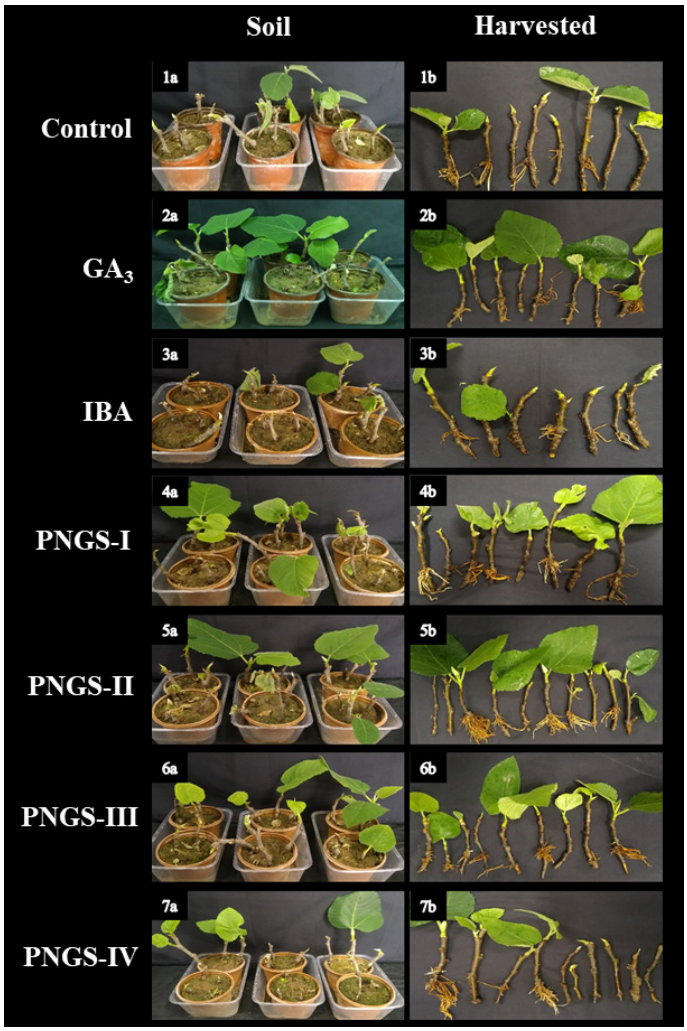

Figure 7: *Fig cultures under different PNGS at 30th day of culturing. (1) Control, (2) GA_3, (3) IBA, (4) PNGS-I, (5) PNGS-II, (6) PNGS-III and (7) PNGS-IV. (a) cuttings in soil and (b) harvested cuttings.*

Roots are the main food collection channels for plants. With the growing areal parts, plants induce more and more roots to enhance the food absorption capacity which fuels the growth process and maintains proportionate development (Kiær *et al.*, 2013). As the root induction is function of auxins, it is evident from the results that IBA application slightly enhanced the

root number as compared to GA_3 and control (Figure 3). PNGS extracts, on the other hand, induced more roots than both the IBA and GA_3. Use of IBA has shown to produce multiple roots in figs (Bayoudh *et al*., 2018; Dhage *et al*., 2015) and our results are testimony to the presence of IBA (or other auxins) in PNGS solutions. Root lengths under PNGS extracts also showed significantly better results, as longer root lengths and were noted for explants under PNGS extracts (Figure 4). Root length indicate soil potentials for Carbon sequestration and for water and nutrient usage (Merrill *et al*., 2002). Once the roots are induced the length is then dependent on the efficacy of the developed root system (Chiatante *et al*., 2018). Ferreira and Pasqual (2005) reported that root elongation can be achieved by coupling GA_3 with an auxin. Bayoudh *et al*. (2018) also reported that IBA supplementation also promotes root lengthening. The results again lead to the deduction that PNGS extracts contain not only the auxin but also the gibberellins as they are maintaining higher root lengths, specifically in PNGS-4.

Survival rate of the *in vitro* propagated plants is dependent on the health, number and length of the developed root systems. On transplantation to the soil the roots have to establish themselves in the new environment and start uptake of the nutrients (Mewar and Naithani, 2016). If the explants have the root system, efficient enough to uptake the nutrients plants are able to survive and develop (Nair *et al*., 2008). The significantly better performance of fig explants under PNGS as compared to standards (Figure 5) is evidence to the presence and balance of growth hormones in these extracts along with other growth promoting substances. As PNGS extracts are raw extracts, they contain all the naturally occurring growth promoting constituents along with balanced nutritional elements that facilitate growth. The availability of a balanced combination of growth hormones and nutrients promote development of rooting parameters which in turn results in a greater survival rate in the field.

Inherent variability of hormonal concentrations may prove to be a limitation to PNGS extracts. The relative concentrations of these substances may vary under different conditions, regions and environmental fluctuations, which may reduce the reproducibility of these extracts along with limiting their potential applications (Pant *et al*., 2021). Pooling the extracts from plants under different conditions and different environments can reduce the variability in the growth hormone concentrations. Furthermore, the source plants exhibit opportunistic growth behavior and aggressive propagation traits, contributing to their extensive biogeographical distribution. This widespread availability enables efficient biomass sourcing for extract preparation, thereby improving the feasibility and cost-effectiveness of large-scale applications

Conclusions and Recommendations

The cultural, economic and nutritional importance of fig makes it pertinent to promote fig conservation and propagation programs. It is necessity of the time to expand these programs at larger scales throughout the country. PNGS extracts showed highly successful and encouraging results for all the rooting parameters and have ascertained their efficacy by developing healthier and robust root systems i.e., earlier primordia formation and roots induction, larger number and longer lengths of roots with higher survival rates. These extracts are not only efficient but they are highly cost-effective and easily available. It is safe to say that the use of PNGS extracts in propagation programs should be promoted so that the full capacity and efficacy of PNGS extracts can be harnessed.

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Novelty Statement

The present study employed Plant Natural Growth Substances (PNGS), marking one the first report of its kind, and demonstrating significant potential for both commercial and research applications. This approach has rendered *in vitro* technology more cost-effective, feasible, and applicable at the consumer level.

Author's Contribution

This research work is part of PhD. research project of Saad Hussain Shah. Asad Jan conceived and designed

the experiments while Israr ud Din was the "Major Lab Supervisor" for the project.

Statement of conflict of interest

The authors have declared no conflict of interest.

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