



An unconventional exploration of axillary buds in Yacon (*Smallanthus sonchifolius*) for planting material production

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ABSTRACT

A protocol was developed for new planting material (NPM) production from axial bud in controlled environment exploring artificial substrate and auxin enriched power in yacon (*Smallanthus sonchifolius*). Yacon, a perennial herbaceous plant from the family Asteraceae was conventionally propagated by propagating roots (corms). The corm production was not fast and easy enough to meet the growing demand for NPM. Aerial stem cutting was an option but it destroyed the mother plant. Seed produced in a plant is of poor quality and most of the seeds are sterile. Thus, assuring the genetic purity, the present innovation identified a continuous and exponential process of NPM production without destruction of the mother plant as well as without sacrificing below ground rhizome. It has potential to produce maximum of 45.16 ± 1.95 NPM per plant. To the best of my knowledge, the production of NPM is being reported from auxiliary bud directly for the first time in yacon.

Keywords: *Smallanthus sonchifolius*, artificial substrate, auxiliary bud, new planting material.

Yacon (*Smallanthus sonchifolius*), a perennial herbaceous medicinal plant, belongs to the family Asteraceae. It is originated from the Andean regions of South America. Yacon root tuber was reported to enrich fructo-oligosaccharides (FOS) and the range was reported from 6.4% to 70% in the dry matter basis and 0.7% to 13.2% in the fresh weight basis. The active ingredient, FOS had been already characterized as an excellent health benefit. It was reported that FOS had a role in reducing glycemic index, controlling body weight, reducing colon cancer, maintaining blood sugar levels, regulating blood cholesterol level, enhancing immune system and moreover, having significant role in body weight maintenance. The FOS enriched yacon tuber as a crop has a potentiality to become a flourishing crop in future as a large number of populations are suffering in health issues. Moreover, its high demand of yacon tubers was already recorded in the market due to rising health consciousness among consumers. Thus, the growing popularity of the yacon tuber was evidenced among aging populations as it was related to overall health care. Yacon is also recognized as an industrial crop for the production of sugar syrup (NRC, 1989). With the increasing demand for yacon in the market due to its medicinal properties, farmers' demand for good quality planting material is also increasing. Therefore, a better, convenient and effective way to achieve mass clonal propagation is a relevant issue. Normally, yacon was conventionally propagated by the propagating roots (corms) but corm

production was not fast and easy enough to meet the growing need for good planting materials among farming community. Thus, rely on corm was not a good option. The reduced flowering emergence as well as subsequent poor fruit set in the cultivated yacon were common problems in *Smallanthus* species (Leon, 1964). Moreover, high proportion of the seeds were evidenced as non-viable and/or of low vigor. Moreover, the seed mediated planting material may not show the genetic purity as it created genetic variation upon crossing. Aerial stem cuttings were also practiced for its propagation if initial environmental condition was fulfilled like reducing desiccation protection (Robinson, 1978; Castañeto and Inhumang, 2004). The major disadvantage of this process is it destructed the mother plant; therefore, this method is destructive and replanting the aerial node may reduce the growth during tuber formation. The present study identified a very simple, low-cost, non-destructive method for NPM production from axial bud. It is also applicable both in controlled environment as well as in open field condition. Thus, huge number of clonally propagated NPM could be possible without destruction of the mother plant in very effective way from the auxiliary bud. This innovation could be adopted by the extension wing to the farming community or growers' community or entrepreneurs' community directly without requiring any costly establishment for the NPM production.

Short Communication

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The yacon plant was planted in the first week of November and maintained in the field located at 28°19'N latitude and 89°23'E longitude and at an altitude of 43 m above the mean sea level. The plant was sown in ridge valley to avoid any excess water. The growing plant characteristically produced axial buds which were explored for new plant production.

Artificial substrate (AS) was formulated with perlite, peat moss and vermiculite (1:1:1) by weight. Perlite was reported for maintaining aeration to ensure an excellent air/water balance which impacts on better root growth including better uptake of nutrients in more effective manner. Peat moss retained moisture for better plant growth which also saves irrigation frequency. Moreover, it releases water and nutrients to the right proportions for optimum plant growth of plant, and reduces the application of manuring. Vermiculite was reported to improve substrate porosity as well as it acted as a medium for water and nutrient exchange. The water soluble NPK (20:20:20) @ 1g per liter of water, phosphate solubilizing bacteria (PSB) and *Trichoderma asperellum* power @5g (2.5g+2.5g each) per 100 ml were used for irrigation in artificial substrate for 2 kg. Both PSB and *Trichoderma asperellum* power were used only once. This AS was known as supplemented artificial substrate (SAS).

The initial environmental incubation was very crucial for new plant emergence. The plastic transparent box with tight lid was used for maintaining the initial humidity. The pot was filled with SAS and kept in transparent box which was again incubated in the environment of 6000 candela steradian meter⁻² (cd·sr m⁻²) light intensity, 14-hour light condition per day maintained in the growth room, 100% of relative humidity. At least, 14 days were followed for initial humidity in the transparent box for assuring the emergence of new plant.

After root emergence from aerial nodal explant recorded at 14 days, the plant was incubated in 6000 candela steradian meter⁻² (cd·sr m⁻²) light intensity, 14-hour light condition per day, 70% of relative humidity in plant growth room (PGR). After further 14 days of incubation, it was directly transferred to open field condition in the early winter season.

The axial bud was harvested from the growing plant and kept instantly in plant growth regulator added water for three hours. The hormone treated axial bud was transplanted into supplemented artificial substrate in the controlled environment and kept for 14 days for further examination.

One-way ANOVA and Tukey's HSD Calculator was used for calculation ([https://www.icalcu.com/stat/anova-](https://www.icalcu.com/stat/anova-tukey-hsd-calculator.html)

[tukey-hsd-calculator.html](https://www.icalcu.com/stat/anova-tukey-hsd-calculator.html)) to calculate p values at 0.05% level of significance to see any significant difference. The MedCalc statistical software (https://www.medcalc.org/calc/comparison_of_means.php) was also explored to calculate the difference between the observed means in two independent samples.

It was found that there were several factors involved for successful axial bud mediated NPM production in the present experiment. First one is artificial substrate formulation which was prepared and autoclaved for maintaining sterility for further infection. The individual component was purchased in sterile condition and formulated accordingly. Further supplementation with the water soluble NPK (20:20:20) @ 1g per liter of water, PSB and *Trichoderma asperellum* power @5g (2.5g+2.5g each) per 100 ml were used for preparation in artificial substrate for 2 kg. Thus, the supplemented artificial substrate (SAS) was prepared from artificial substrate (AS) having only three components like perlite, peat moss and vermiculite. The axial bud was collected from field grown plant and the harvested axial bud was incubated with auxin enriched power (commercially available in the name of 'toto-root' power) to the cut end. The hormone enriched axial bud was further placed on artificial substrate supplemented with NPK, *Trichoderma asperellum* and PSB, known as SAS. The planted axial bud was incubated in closed transparent container for maintaining initial humid condition because failure experiment was evidenced from unlidged box. The axial bud without auxin enriched power was also maintained at the same condition as the merit of the SAS would be evaluated. The transparent box was again kept in plant growth room conditioned with 6000 candela steradian meter⁻² (cd·sr m⁻²) light intensity for 14 hours per day, 22°C temperature. At 14th days, the root was initiated uniformly from nodal region of toto-root (a commercially available auxin enriched powder) incubated axial bud (Fig. 1). The SAS without hormone treated axial bud also showed a few roots but lack of uniformity among all axial bud was observed. Thus, hormone power had a role in uniform initiation of root initiation (Fig. 1). The important observation was that SAS supported the life of the axial bud as none of the axial bud was died. Thus, vitality support from SAS and micro-environment never be ignored.

The effect of toto-root in weight, number of root initiation, highest root length and root diameter at middle portion were also recorded (Fig. 2). At 14th days, no significant initial weight gain was recorded in rooted axial bud treated with hormone enriched power over control as well as harvested axial bud (Fig. 2.A). The number of root initiation was recorded significantly in hormone enriched power (Fig. 2.B). The root length as

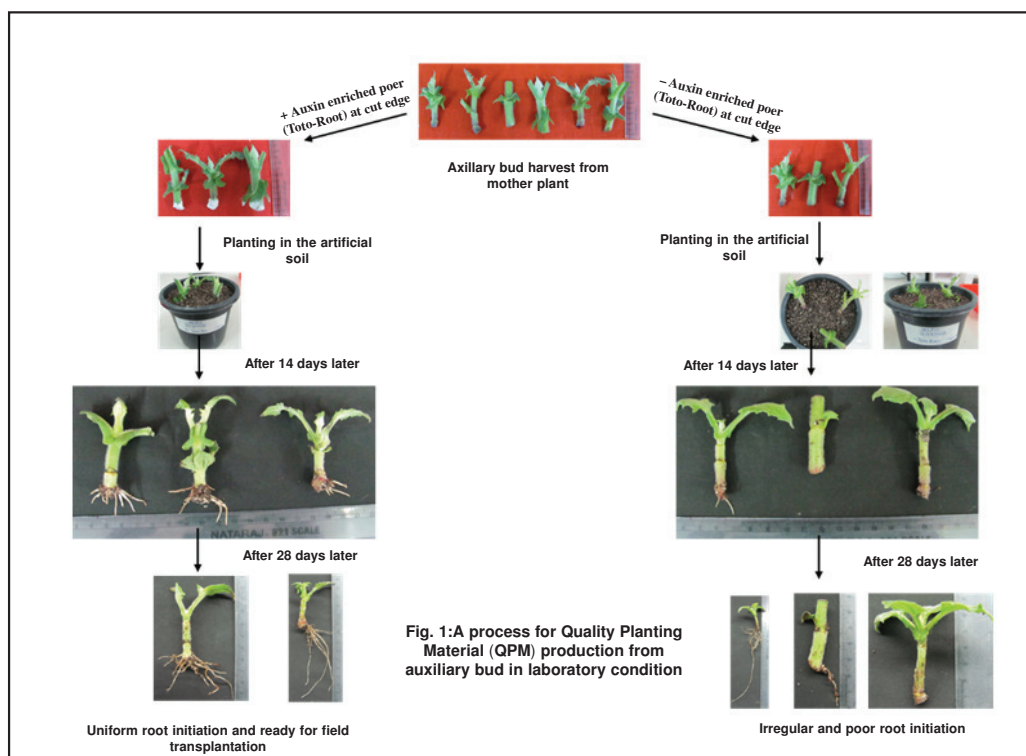


Fig. 1:A process for new planting material (NPM) production from axillary bud in laboratory condition.

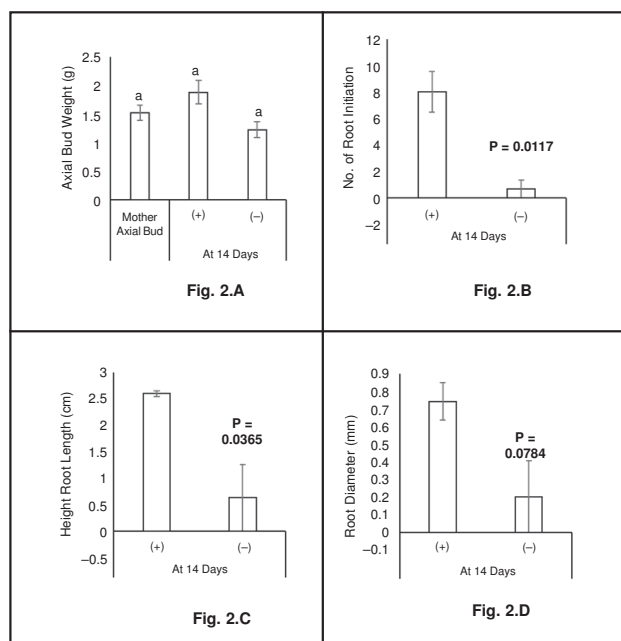


Fig. 2: The effect of hormone enriched power in SAS in weight gain, number of root initiation, highest root length and root diameter at middle portion. **Fig. 2.A.** At 14 days, there was no significant initial weight gain was recorded in rooted axial bud treated with toto-root. **Fig. 2.B.** The number of root initiation was recorded significantly in hormone enriched power incubated axial bud. **Fig. 2.C and D.** The root length as well as diameter at middle of the root were also significantly higher in hormone enriched power incubated axial bud.

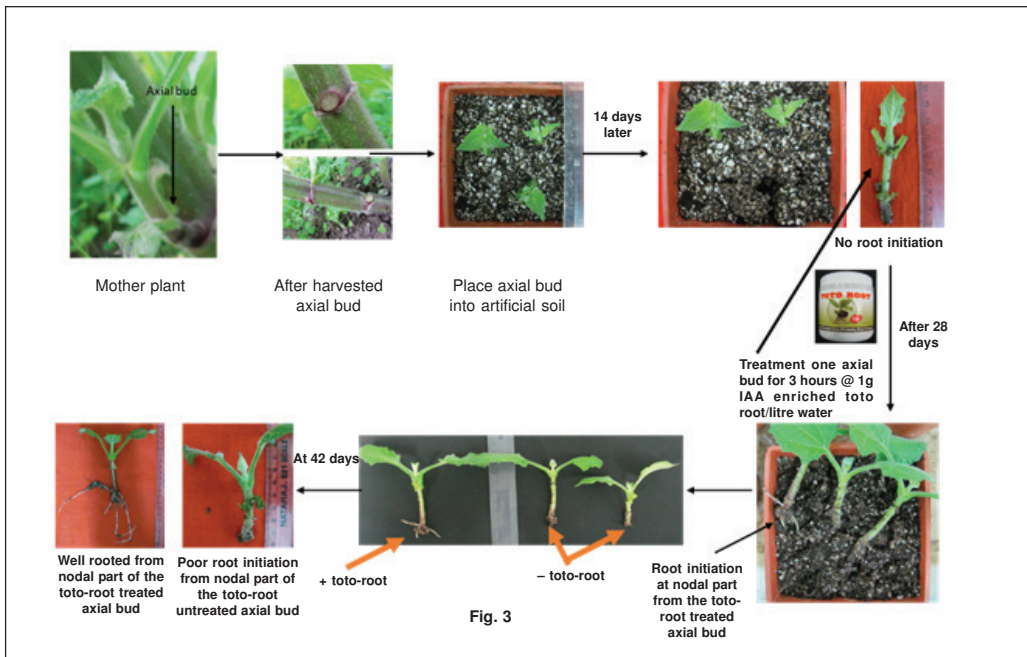


Fig. 3: Role of hormone enriched power in inducing root initiation.

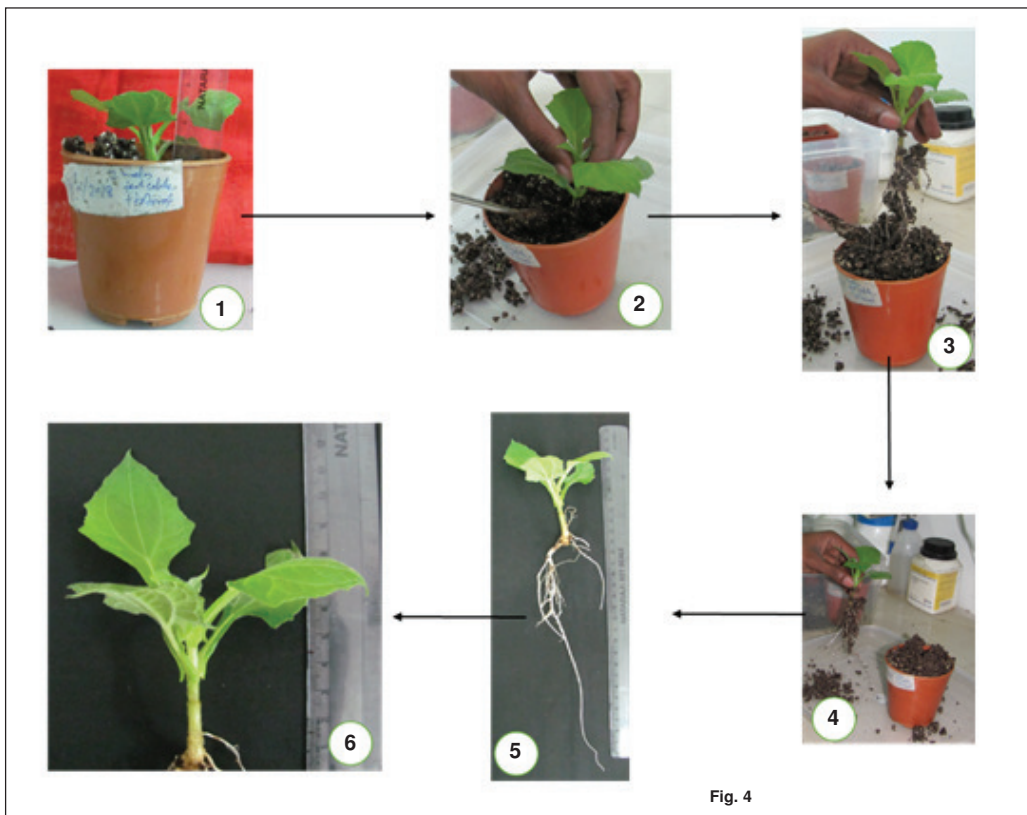


Fig. 4: Pure IAA also induced root initiation from nodal part of the axial bud.

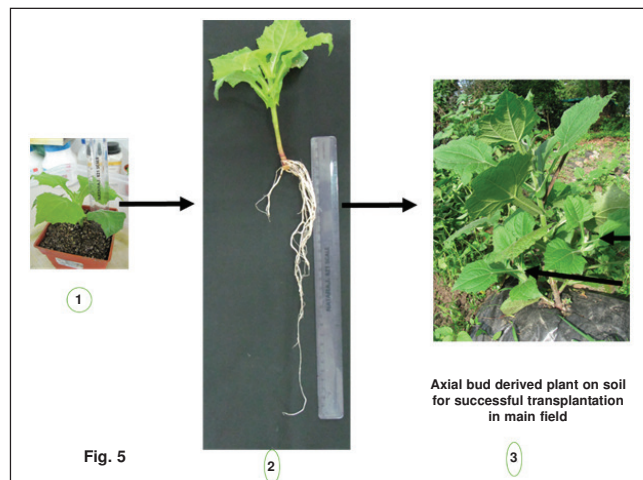


Fig. 5: The 28 days plant derived from axial bud from the laboratory condition and its successful establishment into the main field.

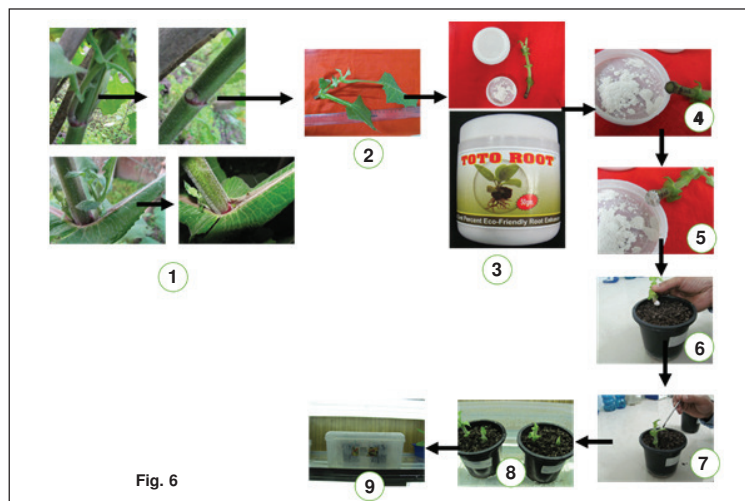


Fig. 6: Process for NPM production.



Fig. 7. The snapshot of the successful standing in the field condition from axial bud derived planting material.

well as diameter at middle of the root were also significantly higher in hormone enriched power (Fig. 2.C and D). The root initiation was evidenced in SAS obviously not in uniform manner. So, the merit of the SAS may be enhanced from further study to develop alternative protocol for NPM production from axial bud.

The specific role of auxin hormone enriched power was also evaluated in the same pot having supplemented artificial substrate (SAS) (Fig. 3). The harvested axial bud from the mother plant was planted into supplemented artificial substrate (SAS) with and without hormone enriched power in the same pot. No root initiation was observed in 14 days after transplanting of the axial bud. At this time, the rootless axial bud was treated with hormone enriched power suspended water solution. After 14 days later or 28 days from the date of experiment initiation, the root initiation was recorded in hormone enriched power treated axial bud. It was mentioned that IAA enriched power @ 1g /litre of water was used for treatment. The hormone enriched power also contained surfactant which helped in adhering hormone to the plant tissue (Fig. 3). Therefore, the hormone enriched power had a role in inducing root from nodal region from the axial bud. For more conformation, pure IAA (HiMedia) was explored for root initiation experiment as the hormone enriched power was purchased from local market and authentication of the presence of specific hormone was needed (Fig. 4). The solution was prepared as one litre from one mg of pure auxin. The root initiation was evidenced at 28 days using the SAS and micro-environment. The IAA incubation to the axial bud was followed @ 1mg IAA per litre of water for 3 hours. Thus, confirmation of root initiation role by the IAA was authenticated. The cost of IAA was very high as compared to hormone enriched power available in the market. Thus, hormone enriched power could be used for uniform root initiation from nodal region from axial bud.

The 28 days plant derived from axial bud from the laboratory condition was transplanted into the main field without any further step for acclimatization (Fig. 5). The axial bud produced by this plant again contributed for further production of NPM and followed a continuous as well as exponential rate of NPM production. Therefore, the easiest process for NPM production could be possible (Fig. 6). The harvested axial bud was touched with hormone enriched power at the cut end and incubation of planted axial plant in humid condition in transparent box in plant growth room conditioned with the micro-environment of 6000 candela steradian meter² (cd·sr m²) light intensity for 14 hours per day,

22°C temperature. At 14th days, NPM could be directly transplanted into field.

The snapshot of the successful standing in the field condition from axial bud derived planting material was presented in Fig. 7. In the lower altitude of the Terai zone, the yacon produced many axial buds which could be explored for NPM production in a continuous and exponential rate. A continuous and exponential rate of NPM production required axial bud production which was evidenced in Terai zone (Fig. 7). Interestingly, the same plant was evidenced in the hill region having less developed axial bud. More research will be required to clarify the axial bud production for variety specific characteristic or altitude specific characteristics. Otherwise, this process will not be relevant for production in a continuous and exponential rate of multiplication in the high altitude. But in the present research, a number of axial bud (average 45.16±1.95) was recorded in the Terai zone.

It was earlier mentioned that the best rooting media for yacon (*Smallanthus sonchifolius*) was reported using its main stem (Angayon *et al.*, 2008). But this main stem detachment was a destructive method as the mother stem needs destruction. From the result, Angayon *et al.* (2008) identified that rooting induction was followed any of the media like pure garden substrate, pure sand, sand and substrate at 1:1 ratio as well as sand and vermicompost at 2:1 ratio. It was further reported that cuttings grown in pure sand had longer adventitious roots compared to the other treatments in all media (Angayon *et al.*, 2008). Tissue culture is an option for producing NPM from any explant or axial bud. But tissue culture required a sophisticated environment like sterile condition and costly equipment. It is required for NPM production, germplasm conservation and soma clonal variation. Moreover, the merit of the tissue culture never be accepted. But aim of the resent study is to develop an alternative method for NPM production. This method should be tissue cultureless, without destruction of the mother plant. Moreover, the tuber, the edible part of the plant will not be sacrificed for the NPM production. The corm produced per plant did not fulfil the demand. Moreover, corm production required a long time, once a year. Axial bud produced by the plant was the target for NPM production. Thus, a method for NPM production was developed from axial bud in the laboratory condition. But this method does not follow any sterilization process, it does no require any fine chemical, of sterile environment as well as any hardening steps. Moreover, 14 days were sufficient for direct field transfer. But a lot of issue will be addressed in the future research like applicability throughout the season or not,

the axial bud production is genetic or environmental as high-altitude plant did not show well developed axial bud. It should be noted that the main purpose was to produce NPM from axial bud, not the yield or performance test. Thus, further research will be required for its performance test specially in term of tuber production. But, to the best of my knowledge, this is the first attempt of NPM production from axial bud directly without destruction of the mother plant in yacon, a medicinally valuable crop.

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