In vitro Conservation of Black Ginger Through Rhizome

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Black ginger (*Kaempferia parviflora* wall ex Baker) is a medium sized genus belonging to the family Zingiberaceae and commercially cultivated in north-eastern part of India including Arunachal Pradesh, Manipur, Meghalaya, Nagaland and Himalayan regions. *Kaempferia parviflora* has been the subject of increased scientific interest in recent centuries. The underground rhizomes are the planting materials in a conventional propagation of black ginger however it has a low multiplication rate (figure 1). Black ginger is a herb that has some historical and medicinal usage for treating metabolic ailments and improving vitality. *Kaempferia parviflora* is health promoting benefits and potential therapeutic functions increases its marketability as herbal products. Despite the high demand for the rhizomes of *K. parviflora*, there is a scarcity of its planting materials. This is due to sluggish natural regeneration of *K. parviflora* through rhizome and a long dormancy period. Under natural conditions *K. parviflora* plants undergo a dormancy phase for five to six months from November to early May during a dry season. The dormancy period affects cropping cycles, year-round cultivation and is a major impediment in the commercial cultivation of this plant. It is known that there are possible methods are available for rapid vegetative propagation of black ginger through direct organogenesis or somatic embryogenesis under *in vitro* conditions but it is necessary to find the best protocol for *in vitro* multiplication of black ginger.

However, significant efforts have been made in the procedure for *in vitro* micro propagation in the other black ginger growing countries. The available literature with respect to *in vitro* plant regeneration has been perused and this article mainly focused on the *in vitro* propagation via direct organogenesis from rhizome buds or shoot tips of black ginger often used as explants. *In vitro* techniques are increasingly being employed to conserve vegetatively propagated crops in various germplasm conservation programs. These are specifically applied to crops where the species are endangered. The main advantage of the technique is the ability to regenerate disease free, true to type plants at high frequency from axillary buds, shoot tips and meristems. Tissue culture is one of the techniques in biotechnology, which has brought about significant impact in the field of plant breeding and conservation of many endangered plants.

In vitro approaches for the conservation and the use of plant germplasm can offer some distinct advantage over alternative strategies. Some of these are as follows: (1) collection may occur at any time independent of flowering period for each species (this assumes that seed material is not required), (2) there is the potential of virus elimination from contaminated tissue through meristem culture, (3) clonal material can be produced where this is useful for the maintenance of elite genotypes, (4) rapid multiplication may occur at any time where stocks are required using micro propagation procedures, (5) germination of difficult or immature seed or embryo may be facilitated for breeding programmes, and (6) distribution across the border may be safer, in terms of germplasm health status using *in vitro* cultures. Some more general positive advantages of *in vitro* techniques include the fact that storage space requirements are vastly reduced compared with field storage. Storage facilities may be established at any geographical location and cultures are not subject to environmental disturbances such as temperature fluctuation, cyclones, insect, pests, and pathogen.

The present investigation deals with collection and *in vitro* conservation of black ginger, slow growth of culture as well as induction of *in vitro* rhizome has been successfully employed for conservation of its germplasm. For the present work, experiments were carried out to study *in vitro* rhizome induction of black ginger, and to study the growth, development and regeneration of the *in vitro* produced rhizomes. So, the work presented here will be beneficial for the

biological conservation as well as for the worldwide pharmaceutical industry as it has also medicinal importance.

Fig. 1. *K. parviflora* growth cycle via conventional propagation: (A) Dormant rhizome; (B) Rhizome with visible bud sprout; (C) Vegetative growth stage of rhizome bud sprouting up to one unfurled leaf stage. This takes approximately ± 120 days. (D) This is a mature adult plant with several unfurled leaves about to start flowering stage at ± 155 days. (E) The true flower of *K. parviflora*. The flowering stage lasts about ± 45 days. (F) At the end of the growth stage ± 240 days, the plant undergoes senescence and the above ground parts dry up as the rhizomes enter dormancy period.

Fig. 2. Different stages of micropropagation in black ginger. (A) Rhizome sprouted bud used as explants for initiation (B) Multiple shoots produced on MS medium containing 6.0 mgl^{-1} BAP (C) *In vitro* rooted plantlets derived from ½ MS medium containing 2.0 mgl^{-1} NAA (D) Acclimatization of rooted plantlets in green house (E) Successfully hardened plants in field

Black ginger micropropagation

To develop axenic cultures of black ginger with rhizome explants were surface sterilized with 100% AgNPs for 60 minutes. Explants from rhizome buds were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of individual plant growth hormone BAP, Kinetin, ADS and 2ip, highest number of shoots was 6.33, highest multiplication rate was 3.33 and highest number of leaves 3.33 were recorded at 6 mgl^{-1} BAP, highest length of shoots 2.7 was recorded on 8 mgl^{-1} BAP. When explants were inoculated on MS medium containing different concentrations and combinations of BAP with NAA, IAA, Kinetin and ADS, highest number of shoots was 5.67 observed on 6 mgl^{-1} BAP + 0.6 mgl^{-1} NAA and 2 mgl^{-1} BAP + 0.5 mgl^{-1} Kn medium, the highest number of leaves was 4.67 on 2 mgl^{-1} BAP + 0.2 mgl^{-1} NAA, highest length of shoots was 2.47 cm and highest multiplication rate (2.67) were recorded on 10 mgl^{-1} BAP + 2.5 mgl^{-1} Kn with three multiplication cycle. Thus, individual effects of BAP improved significantly the shoot growth and proliferation.MS medium supplemented with half strength MS media with 2 mgl^{-1} NAA and gave the highest number of roots (7.56). However, longest roots per explant were obtained with MS + 0.5 mgl^{-1} IAA alone. Plant tissue culture techniques have been applied to produce disease-free planting materials of black ginger to overcome these problems. Hence, the *in vitro*-induced microrhizomes are considered as alternative disease-free planting materials for black ginger cultivation. Therefore, this study was conducted to optimize sucrose and plant growth regulators (PGRs) for its microrhizome induction. Microrhizomes were successfully induced in Murashige and Skoog (MS) medium supplemented with a high sucrose concentration 75 gml^{-1}sucrose). In addition, NAA at 1.4 mgl^{-1} was found more effective for microrhizome diameter, fresh and dry weight. The proliferated shoots were green and healthy in appearance. Finally, healthy and complete plants with

well-developed roots were hardened, acclimatized and planted in the field successfully with a survival rate of 100%.

Conclusion

The present article on *in vitro* shoot multiplication of *Kaempferia parviflora* demonstrates a successful step towards the *in vitro* propagation of the species. Rhizome explants inoculated MS solid medium with 3% sucrose and 0.5 % cleriGar fortified 2.0 mgl^{-1} BA was found be the best protocol for shoot bud induction and multiple shoot production was found on 6 mgl^{-1}BAP. After different experiments, better rooting was observed on half strength MS medium supplemented with 2.0 mgl^{-1} NAA. However, secondary roots observed higher in half strength MS media without any growth hormone. Our developed protocol can also be used to produce a higher amount of large microrhizomes under *in vitro* conditions. The present protocol is a step forward towards an improved commercial propagation system for *Kaempferia parviflora* with more efficiency and multiplication rate than having a rhizome-based propagation.

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