DEVELOPMENT OF MOLECULAR BREEDING TECHNOLOGY FOR PEPPER

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Introduction

Due to asynchronous flowering, fruits of pepper do not mature at the same time. Harvesting is carried out several round of manual picking. This resulted in a high labour output. It is, therefore, desirable to develop pepper genotype with synchronous fruit maturation characteristics so harvesting can be done in one round and probably via mechanisation. This will reduce cost of production significantly. The antisense technology and genetic engineering techniques have been used to develop tomato plants with delayed ripening characteristics. Similar work will be carried out for black pepper. The objectives of the study were: To develop a molecular plant breeding technology for vegetatively propagated crop using black pepper as a model and to apply the antisense technology for the production of delayed ripening characteristic in black pepper.

Materials and Methods

True to type black pepper plant's leaf and stem were used in the regeneration of callus and new axenic plants. Due to the contamination problem encountered using true to type explant, seeds were germinated *in vitro* to obtain axenic plants. Genetic transformation studies are carried out using *Agrobacterium*-mediated system.

Results and Discussion

The highest percentage of explants with multiple shoots after one month was at 0.5 mg/l BAP. The same range of concentration was also carried out with zeatin and kinetin. However, these two hormones did not induce any multiple shoots on stem-nodes. Besides that, callus initiation of leaf, petiole and stem was also carried out using auxins such as NAA, 2,4-D, IAA and Picloram. The concentration used was 0, 0.1, 0.5, 1.0, 2.0 and 5.0 mg/L. From the treatments carried out, all the explants produced callus at different concentration of auxins. Besides producing callus, NAA and IAA also produced roots from the explants especially leaf and stem. Induction of callus from nodal pieces in MS medium was also carried out using NAA at concentration of 0.5, 1.0, 2.0, 3.0 and 5.0 mg/L combined with BAP at 0 and 0.2 mg/L. From the results obtained, all combinations produced callus and in some cases roots were formed in the medium with no added BAP. Calluses subcultured in MS + 0.5 2,4-D + 2.5 kin and kept in dark for about 1-2 month gave quite whitish new callus. Observation on one leaf explant cultured in ½ MS+ 0.5 2,4-D + 2BAP showed direct regeneration after about 2 months. Multiple shoots also observed in stem cultured in MS + 3 IBA + 1.5 BAP after about 2 months. Leaf samples, which have been inoculated with Agrobacterium, were assayed for *gus* activity. The expression of the *gus* gene was indicated by the blue histochemical reaction. So far, *gus* activity was only detected on leaf explants at 10 days after inoculation with a 1:10 dilution of *Agrobacterium tumefaciens*. On the other hand, assay of the npt II gene activity by PCR failed to detect the expression.

Conclusions

Plant regeneration was achieved from true to type explants and *gus* activity was observed in leaf explants indicating transformation has been successfully carried out.

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