ABSTRACT

India is one of the largest rapeseed-mustard growing countries in the world, occupying the first position in area and second position in production after China. After groundnut Brassica (rapeseed-mustard) is the second most important edible oilseed crop in India and cultivated in around 6 million hectares accounting nearly 30% of the total oilseeds produced in the country. When compared to other edible oils, the rapeseed/ mustard oil has the lowest amount of harmful saturated fatty acids. It also contains adequate amounts of the two essential fatty acids, linoleic and linolenic acid, which are not present in many of the other edible oils. The projected demand for oilseeds in India is around 34 million tonnes by 2020, of which about 14 million tonnes (41%) is to be met by mustard alone. Brassica juncea (L.) Czern & Coss a major oilseed crop of Indian sub-continent is considerably hindered by various insect-pests and diseases. The yield losses could be as high as 90% due to aphids (Lipaphis erysimi) alone. Conventional breeding techniques are of limited use as cultivated Brassica normally do not have any inherent resistance against the aphids. The advances in plant genetic engineering in recent years have opened new avenues for crop improvement by introducing resistance genes from other crops into desired crops. Plant lectins, the highly specific carbohydrate binding proteins, are reported to have insecticidal properties. Lectins are currently receiving most interest as insecticidal agents against these sap-sucking insects. When seeds or other plant organs are eaten by predators, lectins come in contact with the intestinal tracts of the predators, possibly inhibiting absorption of nutrients resulting stunted growth of pests and eventually the insect dies. Thus, it is important to isolate lectin gene and transfer them to agronomically important crop plants for developing resistance against aphids for enhancing crop yield.

In the present study, moth bean (Vigna aconitifolia) lectin gene of 826 bp and 843 bp was isolated from moth bean cDNA library and characterized (Acc Nos.JN561787 and JF501650). Lectin gene of 843 bp was used for further study. Primers were designed according to the cDNA sequence and 5’ & 3’ RACE was done to complete the 5’ and 3’ UTR of the lectin gene. RACE and cDNA sequences were aligned and 1086 bp lectin gene was deduced. Finally, 1086 bp lectin gene was amplified with primers. For cloning purpose, primers having restriction sites of KpnI and XbaI were designed according to cDNA sequence and ORF of 843 bp was
amplified with these primers and directionally cloned in binary vector pBinAR under CaMV 35S promoter with nptII/Kn resistant marker and mobilized into Agrobacterium tumefaciens (GV3101) strain for genetic transformation of Brassica juncea (L.) cv. Varuna. The explant stem segments were co-cultivated and placed on medium (MS+2mg/l BAP+0.2mg/l IAA) augmented with cefotaxime (250mg/l) and Kanamycin (25mg/l) and after 20-25 days sub-cultured for further multiplication. The survived green shoots were kept on medium containing IAA (0.2mg/l) for rhizogenesis. The molecular analysis of T0 & T1 transformants with moth bean lectin gene was done by nptII, Southern hybridization, Northern hybridization and RT-PCR for stable integration and expression of the cloned gene. Insect bioassay was done to assess the affectivity of transformants against aphid’s under controlled conditions and the results showed up to 10-15% insect mortality. Therefore, such approach has a potential for developing transgenic crops resistant to insect-pests.