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Cloning and expression of pigeonpea lectin gene in an expression vector and its characterization

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ABSTRACT : The insect pests are causing heavy economic loss to the agricultural production globally. The worldwide use of chemical insecticides and pesticides has increased the cost of pest control and resulted in insecticidal hazards to biological organisms, pollution to the environment and increasing insect resistance. Therefore, the challenge today is to achieve higher and stable crop production with safe and eco-friendly strategies. Plants accumulate a set of defense proteins including lectins. Lectins reversibly and non-enzymatically bind specific carbohydrates and this agglutination property makes them useful against various lepidopteran and homopteran insect pests. The harmful effects of lectins on biological parameters of insects include loss in weight, mortality, feeding inhibition, delays in total developmental duration, adult emergence and fecundity on the first and second generation. Realizing their importance, lectin gene was isolated and characterized from pigeon pea. The isolated pigeonpea lectin (PPL) gene (~825 bp) was first cloned in pENTR-D-TOPO vector, subcloned into an expression vector (Gateway Destination vector pET300/NT-DEST) and transformed into BL21 DE3 pLysS competent cells of *E. coli* for protein expression studies. The PPL gene expression studies were carried out at different temperatures, IPTG concentrations and time intervals. The expression was maximum at 2.0 and 2.50 mM IPTG concentration at 37ºC for 5 hrs. The size of the protein was found to be around ~30 KDa. The expression was confirmed by SDS-PAGE and western blotting. Thus, transferring these defense genes under the control of tissue specific promoters will be an effective tool for sustainable insect pest management programme.

Key Words : Cloning, expression, insecticides, IPTG, lectin, transformation etc.