RESEARCH PAPER

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Putative transgenic plants through *in planta* transformation against *Phytophthora* foot rot in black pepper (*Piper nigrum* L.)

S. ASHA AND P.C. RAJENDRAN

Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Kerala Agricultural University, Vellanikkara, THRISSUR (KERALA) INDIA

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Black pepper (*Piper nigrum* L.), popular as 'king of spices' is one of the important export earning spice crop of India. One of the serious reasons causing the low productivity of black pepper during the last decades was the incidence of foot rot disease caused by the *Phytophthora capsici* in the pepper plantations of Kerala, the major pepper producer state in India. Since all the available cultivated varieties are susceptible to this disease, an attempt was done for utilising the disease resistance available in the related pepper species by adopting a different plant transformation approach. The *in planta* transformation via pollen tube pathway was done in the black pepper variety Panniyur-2, using the total exogenous DNA of *Piper colubrinum* , a wild relative species of Piper resistant to the dreaded foot rot disease caused by *Phytophthora capsici*. The resulting putative transformant seeds were germinated *in vitro* by embryo rescue technique. These germinated putative transformants were later subjected to *in vitro* multiplication, elongation and rooting. These cultures were screened *in vitro* in the rooting phase by incorporating the toxic culture filtrate of the pathogen *P. capsici* in the rooting media. The rooted putative transformant plantlets were hardened and screened artificially for disease tolerance under ex vitro conditions. The survived seedlings were planted out. The RAPD analysis of the plantlets with the decamer primers OPA 08 and OPG 08 have shown variation in banding pattern compared to the DNA recipient parent *P. nigrum* variety Panniyur-2.

Key words : Black pepper, *Piper nigrum*, *Piper colubrinum*, *In planta* transformation, Pollen tube pathway transformation, *In vitro* germination, Embryo rescue, Disease screening, RAPD analysis.

INTRODUCTION

The dried mature seeds of black pepper, known as the king of spices or black gold is the most widely used spices in the world. The projected world consumption of black pepper is estimated to be around 2.3 lakhs and 2.80 lakh metric tons by 2010 and 2020, respectively. One of the major reasons for the perceptible decline during the last few years of pepper production is the foot rot disease of black pepper caused by the pathogen *Phytophthora capsici*. To bridge the gap between demand and consumption a modest hike of 1.0 lakh metric tons is required for the next two decades by large scale cultivation of tolerant or resistant cultivars against the biotic stresses especially *Phytophthora* foot rot diseases (Ravindran, 2000). All *Piper nigrum* cultivars are susceptible to this serious disease and have to be chemically protected. The demand for clean spices free from chemical residues and attempts to reduce the cost of cultivation led to intensive research for host-plant resistance. *Piper colubrinum*, known as Brazilian thippali and a distantly related species from South America, is resistant to *Phytophthora* (Ravindran, 2000). However, natural genetic barriers have limited the resources available from other species.

Genetic transformation is a useful approach to overcome the problem of sexual barriers and to introduce desirable genes from other sources into plants for cultivar development. The unique *in planta* transformation method to produce high frequency genetic transformation by applying exogenous DNA to pollen grains and to stigma at the time of pollination is more adequate in this aspect. This approach may be imitating the conventional hybridization with a touch of advance biotechnology. The repeated *in planta* transformation using F_1 putative transformant like back cross method may be helpful to evolve *Phytophthora* foot rot resistant variety of black pepper. Increased selection pressure through *in vitro* technique may also be advantageous in confirming the tangible result in the perennial spice crop like black pepper. In the present study, an *in planta* transformation was attempted in *Piper nigrum* var Panniyur-2 by adopting pollen tube pathway transformation using total exogenous DNA of *P. colubrinum*. Panniyur-2 is the open pollinated variety selected from Balankotta.

MATERIALS AND METHODS

Six bush pepper plants of variety Panniyur-2 (3 years old