วารสารเกษตร 10(3) : 209 - 215 (2537)
Journal of Agriculture 10(3) : 209 - 215 (1994)

Selecting Diploid Potato Clones Suitable for Transposon Tagging of the R-genes against Late Blight (*Phytophthora infestans*): Selecting Genotypes Carrying R_1 and R_3

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Abstract : The process of developing and selecting diploid potato (*Solanum tuberosum* L.) clones for transposon tagging of the *Phytophthora infestans* resistance genes-R. was applied to various potato genotypes. The plants carrying allele R_1 or R_3 were selected for possessing the following characteristics: diploid genome, high fertility, straight segregation of the R-allele, efficicient regeneration *in vitro* and high rate of transformation by the *Agrobacterium tumefaciens* carrying genes NPTII and GUS interon. Three of each R_1- and R_3-carrying clones performed those desirable characteristics.

Index words : potato, late blight, *Phytophthora infestans* , breeding for resistance, genetic transformation

Introduction

Genetic transformation of plants has become a major topic among all genetic improvement of plants and soon far more progressed beyond the conventional counterpart during the pass two decades. The genetic transformation specifically and efficiently introduces foreign gene into certain plant genome regardless the species of origin. Although there are many ways of genetic transformation, however, microbial-mediated one has been intensively employed. T_i or R_i plasmid of a bacterium genus *Agrobacterium* functions as a carrier to transfer the gene of interest into the target plant genome *via* the process of infection (Draper *et al* , 1988). The transferred gene expresses itself normally as it does in the original species and can be

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detected as traits or chemical reaction of the so-called 'marker gene' in the host tissue. Such marker gene is, for example, drug or antibiotic resistance gene which can be screened by culturing the host tissue in medium containing the drug *in vitro*. Susceptible genotypes will be killed while resistant ones survive. In this gene tagging scheme, a marker gene employed is categorised in a group of 'transposable element' or **transposon** : a chromosomal fragment that can insert itself into certain chromosome arrangements thus inhibits the gene expression. These transposons are **Ac** and **Ds** elements of maize found in 1945 by McClintock. (McClintock, 1945). They will tag for the R-gene, resistance gene of potato against late blight, of which its protein products are unknown. In the second step of the scheme, splicing and manipulating of the R-gene will be made possible. This study aimed to select a group of potato genotypes carrying a particular R-allele and required characteristcs that facilitate the R-gene tagging.

Material and methods

Potato (*Solanum tuberosum* L., 2n = 4X = 48) cultivars carrying R_1 or R_3 allele were diploidized by crossing with a dihaploid pollinator, *S. phureja* cv. IVP 101 (Hutten *et al.*, 1990). The dihaploid progenies, characterized by purplish spots of anthocyanin accumulation were collected as described by Hougas and Peloquin (1958). The dihaploids were rechecked to ensured ploidy level by counting chloroplast number in stomatal guard cells as described by Jacob and Yoder (1989). Detached Leaflet inoculation was employed to screen late blight resistant plants according to Toxopeus (1954) with modifications (Lapwood, 1961; Mooi, 1965; Umaerus, 1969ab; and Umaerus and Lihnell, 1976). Defined *P. infestans* pathotypes were courteously provided by the Institute for Plant Protection, Wageningen. The genotypes performing hypersensitive resistant : susceptible ratio approaching 1:1 were kept for futher study, otherwise discarded. Flowered resistant dihaploids were crossed with fertile diploids, ie. IVP 1024-2, SUH 3711, IVP 1031-29 or IVP 1030-9, to improve fertility and vigor. Diploid progenies were rescued according to Singsit and Hanneman (1991) and Neal and Topoleski (1983). This step was repeated if the result was unsatisfiable. Derived diploid progenies were brought from *in vivo* to *in vitro* following Old and Primrose (1989). Axenic diploid plantlets were multiplied in 8-cm. jars of MS medium (Murashige and Skoog,(1962), 30gl $^{-1}$ sucrose, 8 gl $^{-1}$ agar) .

Testing for regeneration ability was done as described in Visser *et al* (1989 ab) and Visser (1991); stem segments were cultured on ZCV medium (MS inorganic salts, 1 mgl $^{-1}$ zeatin, 200 mgl $^{-1}$ claforan, 200 mgl $^{-1}$ vancomycin, 30 gl $^{-1}$ sucrose, 8 gl $^{-1}$ agar). Meanwhile transformation test was done the same way but the stem segments were soaked in culture suspension of

A. tumefaciens strain LBA4404 (Hoekema *et al*, 1983) carrying plasmid p35SGUS-interon and NPTII gene (Vancaneyt *et al*, 1990) then transferred on the selection medium. The expression of the plasmid was resistance to kanamycin and production of enzyme B-glucuronidase (GUS). The transformation method was modified from Visser (1989 and 1991) as described in Thaisantad (1992). The selection medium was ZCV with 100 mgl^{-1} kanamycin. The B-glucuronidase histological assay was based on Jefferson *et al* (1987).

Results and Discussion

Five R_1 and 9 R_3 diploids were obtained after repeated crossing to reduce ploidy level and improve vigor. Those diploids performed much better compared with their dihaploids in terms of fertility and vigor which was found also in the study of Hougas and Peloquin (1958). The histological reaction of GUS test was seen as blue spots on the cut surface of 10-day-old explants where callus tissue initiated. This result is resemble to Jefferson *et al* (1987). After testing for regeneration ability, expression of kanamycin resistance and activity of B-glucuronidase, the result is summarized in table 1.

From table 1, among R_1 clones, J92-6448-2 performs the best when compared with other genotypes. It shows high rate of regeneration (12.5% of the explants produced shoots), high GUS reaction (5 positive spots) and moderate kanamycin resistance (18.91% viable calli on selection medium). On the other hand, J92-6448-10 shows highest GUS reaction (33 positive spots) and kanamycin resistance (68%) but no shoot was differentiated. Nevertheless, it produces viable calli in high rate (35 explants with callus/40 total explants, data not shown). J92-6448-11 shows high rate of GUS reaction (5 positive spots) though low rates of kanamycin resistance (4% viable calli) and regeneration (14% shoots). For the rest, J92-6448-5 and J92-6448-7, their performance is poor in all the tests.

For R_3-clones, J92-6453-9 shows high GUS reaction (11 spots), high kanamycin resistance (37.5% viable calli) though low regeneration rate (7.5% shoots). On the other hand, J92-6449-6 shows high kananmycin resistance (26.31% viable calli), high regeneration rate (22.5% shoots) but low GUS activity (1 spot). Unfortunately, in J92-6450-5, GUS reaction is high (5 spots) but kanamycin and regeneration rates are nil. For the rest of R_3 group the rates of kanamycin resistance and GUS reaction are low though some have considerably high regeneration rate.

Table 1 Transformation (kanamycin resistance and GUS positive reaction) and regeneration (% number of shoots/explant) rates of R_1 and R_3 clones.

clone	kanamycin+/- [1] (%)	GUS+/- [2] (spot)	% number of [3] shoots/expl.
R_1			
J92-6448-2	18.91	5	12.50
J92-6448-5	0	0	0
J92-6448-7	7.14	0	0
J92-6448-10	68.00	33	0
J92-6448-11	4.00	5	2.50
R_3			
J92-6449-1	0	1	0
J92-6449-6	26.31	1	50.00
J92-6450-5	0	5	0
J92-6453-6	0	0	22.50
J92-6453-9	37.50	11	7.50
J92-6454-37	0	0	0
J92-6454-38	0	0	5.00
J92-6454-40	0	0	15.00
J92-6454-47	0	1	55.00
control			
IVP 1024-2	0	2	17.50

[1] = percent number of explants with callus survived per total (4-37) explants

[2] = number of GUS positive reaction spots per 5 explants tested

[3] = percent number of shoots per total (40) explants

Discussion

GUS reaction and kanamycin resistance are, in principle, the indication of successful transformation. Theoretically, the genotype that shows positive GUS reaction should also shows high rate of kanamycin resistance because these genes are linked (Hoekeme *et al*, 1983). From the result, some genotype did not follow this theory, for example, J92-6449-1, J92-6450-5, J92-6454-47 of the R_3 group shows GUS reaction but no kanamycin resistance. Even in the control, IVP 1024-2, which always shows high rates of GUS and kanamycin reaction, also has low kanamycin resistance. There are two explanations for this phenemenon. Firstly, testing of kanamycin resistance and GUS reaction was done independently. Moreover the sample size was small so there was higher chance that some explants escaped the infection of *Agrobacterium tumefaciens*. Secondly, there might be some amount of kanamycin left from the initial inoculum of *Agrobacterium*, therefore kanamycin did not inhibit only susceptible explants but also do to the resistant ones. The problems can be overcome by increasing the sample size to 40 or more and the initial inoculum of *Agrobacterium* must be rinsed by resuspending in fresh medium without kanamycin and recentrifugating the cuture 2-3 times prior to inoculation.

In conclusion, the genotypes that performed chracteristics facilitating for further R-gene tagging, from the most to the least are, J92-6448-2, J92-6448-11, J92-6448-10 for R_1 group, and J92-6449-6, J92-6453-9, and J92-6454-47 for R_3. In the next step of this scheme, transposon tagging for the specific R-alles can be done in homozygotes (RR) of these lenghtily developed genotypes.

Ackowledgement

I wish to extend my gratitude to Professor E. Jacobsen and Mr. A. El-kharbotly from the Department of Plant Breeding, Wageningen Agricultural University, Wageningen, the Netherlands, for their patient supervision on this research.

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