Segregation of isozymes in selfed progenies of a synthetic amphidiploid between *Solanum integrifolium* and *S. melongena*

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Summary

Isozyme and cytogenetic analyses were performed on selfed progenies of a synthetic amphidiploid between scarlet eggplant, *Solanum integrifolium* (= *S. aethiopicum*), and eggplant, *Solanum melongena* ‘DMP’, for estimating genetic uniformity. Isozymes in the 379 examined seedlings segregated into five genotypes (phenotypes) each at the four loci examined, *Pgd-2* of phosphogluconate dehydrogenase (E.C.1.1.1.43), *Idh-2* of isocitrate dehydrogenase (E.C.1.1.1.41), *Pgm-2* of phosphoglucomutase (E.C.2.7.5.1) and *Skdh-1* of shikimate dehydrogenase (E.C.1.1.1.25), indicating that the selfed seedlings were not genetically uniform. Most of the examined 15 selfed seedlings exhibited a somatic chromosome number of 48, that is the same number of the synthetic amphidiploid, whereas isozyme genotypes among them were variable. It is suggested that the segregation of isozymes was not caused by variation of chromosome number but by genetic segregation of isozyme genes. The genome of the synthetic amphidiploid was indicated to be unstable.

Introduction

Commercial production of eggplant (*Solanum melongena* L.) has been limited by bacterial wilt, *Fusarium* wilt and some other soil-borne diseases (Khan et al., 1978; Mochizuki & Yamakawa, 1979; Yamakawa & Mochizuki, 1979; McCammon & Honma, 1983; Ali et al., 1990). Scarlet eggplant, *Solanum integrifolium* Poir. (= *S. aethiopicum* L.) and its hybrids with eggplant are highly tolerant to some pathogenic strains of *Pseudomonas solanacearum* (Ali et al., 1990) and *Fusarium* wilt (Yamakawa & Mochizuki, 1979). Efforts to transfer the resistances in *S. integrifolium* into cultivated eggplant have been intensively performed without success because the hybrids were highly sterile (Khan et al., 1978; Nishio et al., 1984; Ali & Fujieda, 1990). Direct use of the interspecific hybrids as rootstocks might have considerable importance, however, hybrid seed production needs immense labor, such as manual emasculation, hand pollination etc. (Ali et al., 1992).

Recently, we have succeeded in restoring fertility of a commercial rootstock cultivar ‘Assist’, *F₁* hybrid of *S. integrifolium* × *S. melongena* ‘DMP’, by chromosome doubling (Isshiki, 1996). This chromosome doubled plant, a probable amphidiploid, produced a large number of seeds without hand pollination. These seeds are expected to be utilized for efficient propagation of ‘Assist’.

In the present study, genetic uniformity of the selfed seedlings of the synthetic amphidiploid of ‘Assist’ was examined by isozyme and cytogenetic analyses to know whether the amphidiploid can be utilized for producing uniform seeds of ‘Assist’. Further, genetic stability of the genome of the synthetic amphidiploid is discussed.

Materials and methods

The plant materials used were ‘Assist’ (*S. integrifolium* (= *S. aethiopicum*) × *S. melongena* ‘DMP’) its
parents, a synthetic amphidiploid of ‘Assist’ and 379 seedlings obtained by selfing of the amphidiploid.

Isozymes at four loci, Pgd-2 of phosphogluconate dehydrogenase (PGD, E.C.1.1.1.43), Idh-2 of isocitrate dehydrogenase (IDH, E.C.1.1.1.41), Pgm-2 of phosphoglucomutase (PGM, E.C.2.7.5.1) and Skdh-1 of shikimate dehydrogenase (SKDH, E.C.1.1.1.25), were used for examining genetic uniformity of the selfed seedlings since these isozymes were known to be polymorphic between S. integrifolium and S. melongena (Isshiki et al., 1994a). Enzymes were extracted from young developing leaves of the plant materials according to Wendel (1983) and separated electrophoretically on either vertical polyacrylamide slab gels or horizontal starch slab gels. Polyacrylamide gel electrophoresis was employed for the separations of PGD and SKDH isozymes. Five percent running gels (Tris-HCl pH 8.9) and 4.2% stacking gels (Tris-HCl pH 6.7) were used. Tris-glycine pH 8.3 buffer was utilized as the electrode buffer. Gels were run at 15 mA constant amperage until the marker dye (bromophenol blue) migrated to the end of the gel. Starch gel electrophoresis was used for separating IDH and PGM isozymes according to the procedures described by Wendel & Parks (1982) and Wendel (1983). Starch gels were run at 25 mA constant amperage for 6 h. All the electrophoreses were carried out at 4 °C. Enzyme assays for the four enzymes were conducted as described by Wendel (1983).

Nomenclature for isozyme alleles at the four loci in eggplant and its wild species was described in previous papers (Isshiki et al., 1994a; Isshiki, 1996). However, in the present study, the allele originating from S. integrifolium was assigned for convenience as i (the initial letter of S. integrifolium) and that from S. melogena ‘DMP’ as m (the initial letter of melongena) at each of the four loci. Based on the findings obtained by genetic analyses in the previous studies (Isshiki et al., 1994b; Isshiki, 1996), isozyme genotypes at the examined four loci were assumed. The difference of relative intensities of the isozyme bands due to gene dosage effect of the alleles was also assessed to assume the genotypes.

Root tips of the synthetic amphidiploid and 15 selfed seedlings, which were randomly selected from the 379 selfed seedlings mentioned above, were pre-treated with a 0.002 M 8-hydroxyquinoline for 2 h at 20 °C and fixed in ethanol/acetic acid solution (3:1 v/v). Somatic chromosomes in cells of these root tips were observed by the Feulgen staining method.

Chromosome associations at metaphase I (M I) of the synthetic amphidiploid were observed in smear preparations of 20 pollen mother cells (PMCs) from fresh anthers stained in acetic carmine.

Results

Segregation of isozymes at the examined four loci, Pgd-2, Idh-2, Pgm-2 and Skdh-1 was observed in the selfed seedlings of the synthetic amphidiploid. With Pgd-2 (Figure 1), ‘Assist’ and its synthetic amphidiploid exhibited the same triple-banded phenotype, i.e., the both parental bands of equal staining intensity. Their genotypes were assumed as im for ‘Assist’ and imm for the amphidiploid. The selfed seedlings showed five kinds of isozyme phenotypes, i.e., two single-banded phenotypes and three triple-banded ones. The single-banded phenotypes were the same as those of ‘DMP’ or S. integrifolium, therefore, the genotype of the former phenotype was assumed as mmmmm and that of the latter one as iii. The triple-banded phenotypes contained three types, 1) the same phenotype of the synthetic amphidiploid, 2) the upper two bands stained more intensely than the lower one and 3) the lower two bands stained more intensely than the upper one. Since these differences among the three triple-banded phenotypes could be explained by gene dosage effects for four allelic doses, their genotypes were assumed as iimm, immmm and iimm, respectively (Figure 1).

The isozyme genotypes at the remaining three loci could be also assumed by similar interpretations performed for Pgd-2, since the asymmetry of the relative intensities of the isozyme bands could be recognized clearly at the three loci (Figures 2–4). Although there was the tendency that the isozyme band originating from S. integrifolium showed higher intensity than that from ‘DMP’ for Skdh-1, this did not prevent their genotypes from being determined.

Isozymes at the four loci segregated into five genotypes (phenotypes) in the selfed seedlings (Figures 1–4). Segregation did not follow a tetrasomic pattern, 18:1:1:8:8 (= iimm:iiii:mmmm:iimm:iimm) due to a marked excess of genotype iimm in the progeny plants (Table 1).

All the randomly selected 15 selfed seedlings, except one, exhibited a somatic chromosome number 48, the same as that of the amphidiploid, although isozyme genotypes among them were variable (data not shown).