Theor Appl Genet (1990) 80:390–394

THEORETICAL AND APPLIED GENETICS

Somaclonal variant UC-T3: the expression of *Fusarium* wilt resistance in progeny arrays of celery, *Apium graveolens* L.

S. Heath-Pagliuso and L. Rappaport *

Department of Vegetable Crops, Plant Growth Laboratory, University of California, Davis, CA 95616, USA

Received October 26, 1989; Accepted April 23, 1990
Communicated by A. L. Kahler

Summary. First generation (S_1) progeny, second generation (S_2) progeny, and backcross (BC) progeny of a celery (*Apium graveolens* L. var. *dulce*) somaclonal variant, UC-T3, were evaluated for resistance to the fungus *Fusarium oxysporum* f. sp. *apii*, race 2 (FOA_2). Chi-square analysis of S_1 progeny showed that the expression of resistance did not fit a single-locus model. S_2 progeny means were similar among families, except in a heavily infested field. The lowest ranking S_2 family in both the lightly infested and heavily infested fields was significantly more resistant to FOA_2 than individuals of the susceptible progenitor line 'Tall-Utah 527OR'; therefore; it was concluded that the trait was heritable. The phenotypic distribution of the backcross progeny was broad, suggesting that the new resistance was conferred by at least two genes whose expression was dominant to susceptibility. The mean scores for disease resistance of the progeny of crosses between UC-T3 and the moderately resistant line, 'Tall-Utah 527OHK', generally equaled the resistance found among the progeny of the most resistant parent.

Key words: Somaclone – *Fusarium oxysporum* f. sp. *apii* – Tissue culture – Fusarium wilt – *Apium graveolens* L.

Introduction

Cell and tissue culture can be very useful for creating new genetic variation (Larkin and Scowcroft 1981; Lee and Phillips, 1988). Much of the variation is genetic and heritable (Earle et al. 1978; Evans and Sharp 1983; Larkin et al. 1984; Orton 1984; Scowcroft et al. 1983; Zong-xiu et al. 1983), affecting single and polygenic characters (Evans and Sharp 1983; Evans et al. 1984; Larkin et al. 1984; Shepard 1981; Singer and McDaniel 1984), and organelle (McNay et al. 1984) as well as nuclear genomes.

Although there has been considerable interest in somaclonal variation, very few crop cultivars have been derived from tissue culture with novel disease resistant germplasm (Daub 1986). Probable reasons are that not all crop species are amenable to cell culture, that it is often difficult to select for resistance at the cell level, and that it may be necessary to regenerate plants prior to selection, a procedure that involves much time and labor to achieve an uncertain outcome. Furthermore, as with any new source of germplasm, the resistance must be fully characterized to determine the most appropriate method of incorporating it into existing varieties.

Celery (*Apium graveolens* L. var. *dulce*) has many natural pathogens. It is particularly susceptible to *Fusarium* wilt, caused by the fungus *Fusarium oxysporum* f. sp. *apii*, race 2 (FOA_2). Conventional breeding methods to introgress genes for resistance in celery are very time-consuming. Moreover, when we began this research no resistant material was available for breeding. Cell culture seemed a viable alternative to conventional breeding methods, because celery is relatively easy to culture and regenerate (Chen 1976; Williams and Collin 1976; Al-Abta and Collin 1978; Dunstan et al. 1982; Rappaport et al. 1988; Nadel et al. 1989) and selection procedures using regenerated plants to obtain resistance to FOA_2 and other diseases seemed promising (Rappaport 1982; Pullman and Rappaport 1983; Heath-Pagliuso et al. 1988; Wright and Lacy 1988). Wright and Lacy (1988) showed that regenerated plantlets from two celery lines ranged from highly susceptible to highly resistant for three fungal pathogens and one bacterial pathogen. Our results showed that the progeny of a somaclonal variant of cel-

* To whom correspondence should be addressed

ery, UC-T3, obtained from a FOA_2-susceptible cultivar, 'Tall-Utah 527OR' (527OR), had increased resistance to FOA_2 (Rappaport and Heath-Pagliuso 1986, 1987; Heath-Pagliuso et al. 1988). In order to fully characterize this germ plasm, we have compared S_1 and S_2, backcrossed (BC_1) and outcrossed progeny arrays for level of resistance and stability. Inferences were made as to the putative genotype of UC-T3, as well as to the usefulness of this new germplasm.

Materials and methods

Plant material

Seed of the FOA_2-susceptible line, 'Tall-Utah 527OR' (527OR), and the moderately tolerant 'Tall-Utah 527OHK' (527OHK), were supplied by Sun Seeds Inc., CA. 527OR was used as the parental material to establish cell culture lines, because it was the most commonly grown cultivar in California at the time these studies were initiated. Because the parent of UC-T3 and all its siblings died as a result of FOA_2 infection, 527OR plants from commercial seed were used as an indication of the resistance present in the original parent. Tests were conducted either in the greenhouse or in the field.

Tissue culture and origin of progeny generations

The tissue culture methods and the origin of the somaclonal variant UC-T3 have been reported elsewhere (Heath-Pagliuso et al. 1988). UC-T3 was self-pollinated to yield the first generation progeny (S_1). Second generation progeny (S_2) were obtained by self-pollinating S_1 individuals that survived to maturity following exposure to *Fusarium*. Backcross progeny (BC_1) were obtained by crossing S_1 plants that survived *Fusarium* wilt treatment to 527OR. Another cell culture line, HK-15, was initiated from 527OHK and regenerated plantlets were evaluated for resistance in the greenhouse. At maturity, plants that received a rating of zero were self-pollinated or were crossed to S_1 progeny of UC-T3.

Barley straw inoculum

Forty grams of barley straw inoculum was prepared by mixing 10 ml of *Fusarium oxysporum* f. sp. *apii,* race 2 (FOA_2) microconidial suspension (10^6 spores/ml) grown in Charudattan media (Charudattan and DeVay 1972) following the method of Schneider (1984). Three-month-old greenhouse-grown plants were transplanted to soil (UC mix) containing 5 g of barley straw inoculum per kilogram of soil.

Disease evaluation

Plants were evaluated for disease by slicing the root and crown tissue. Each plant was assigned a root score based on visual determination of the amount of vascular discoloration and decay characteristic of FOA_2 infection. We initially rated the plants using a scale of 0 (healthy – no decay or discoloration) to 7 (dead), but changed to an index of 1 (healthy) to 5 (dead) to conform with the index used by other researchers studying FOA_2 resistance. Mean root score (MRS) is the average of the root scores of all the progeny tested per family or per cross. Field-grown plants were examined at 90 days and greenhouse trials were conducted 10 weeks postinoculation. Scoring the roots in this manner does not destroy the plant. Thus, those plants receiving a zero rating could be transplanted and allowed to reach maturity in the greenhouse.

Experimental design

In greenhouse trials the plants were completely randomized. Field trials were conducted in farmer's fields by the local farm advisor or seed company representative and were necessarily restricted by the cultural and harvesting practices of the grower. At each location, several lines being tested for FOA_2 resistance, ours included, were planted in rows in a small quadrant of the field. A commercial crop was usually planted in the adjoining rows. The lines were randomized between locations. Due to the small number of seeds available and a desire to test celery grown in both non-FOA_2- and FOA_2-infested fields, the lines were not replicated within a location.

Statistical analyses

All statistical analyses were performed using the Stats Plus Program for the Apple IIe (Human Systems Dynamics). Analysis of variance was used to test for significant differences among the means. Student's *t*-test was used to determine ranking (Steel and Torrie 1960).

Results

Selfed progeny

The S_1 progeny were evaluated in the greenhouse and the data fit a bimodal frequency distribution (Fig. 1). Two models that give a bimodal distribution are the single gene dominant (3:1) and the two-locus model with both genes dominant (9:7). Chi-square analysis shows that the data are not significantly different from what is predicted by the two-locus model (Table 1).

UC-T3 S_1 progeny and the S_2 progeny of one family were tested for resistance to FOA_2 (October, 1986). The progeny means of both generations were significantly different from 527OR at the 1% level of significance; therefore, an additional 12 families (S_2 progeny) were tested in two fields, A and B, in Oxnard the following spring (Fig. 2, Table 2). The fields differed in the degree of FOA_2 infestation, as shown by the different ratings obtained for the susceptible cultivar, 527OR. In field A, which had the lowest level of FOA_2 infection, there were no differences among means. In contrast, significant differences were observed among families in field B. In both fields, however, even the lowest ranking family was significantly healthier than individuals of 527OR (Table 2).

Backcrosses

Backcrosses of UC-T3 S_1 individuals to 527OR were tested on a farm in Salinas and at the South Coast Field Station in Orange County. A wide range of MRS was obtained, depending upon the parents used in the crosses (Table 3). However, the overall MRS of the progeny of all crosses were significantly lower (the plants were healthier) than those for 527OR and comparable to, or in one case significantly lower than, the tolerant cultivar, 527OHK (Table 3).

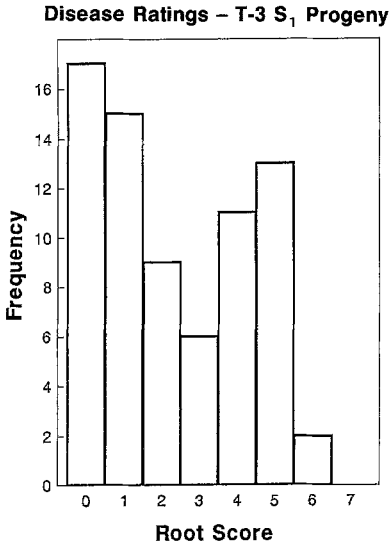

Fig. 1. Frequency distribution of root scores for S_1 progeny of UC-T3. Plants were evaluated for presence of disease in the greenhouse after 10 weeks in soil containing 5 g inoculum/kg soil. The rating scale ranged from 0 (healthy) to 7 (dead)

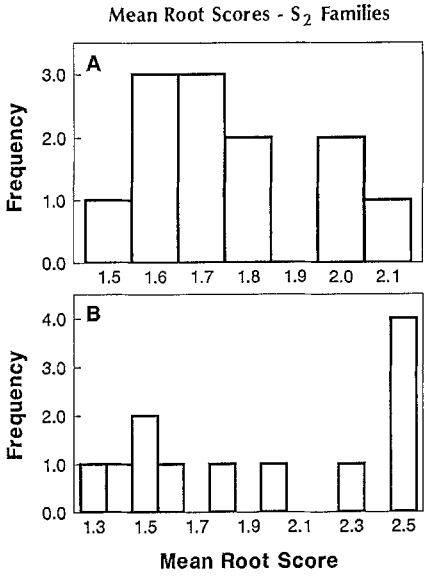

Fig. 2. Frequency distributions of mean root scores for the members of each of 12 UC-T3 S_2 families in two field trials, A and B, in Oxnard, CA. Mean root scores for the control, 'Tall-Utah 527OR', were 3.4 and 4.0 in A and B, respectively. The rating scale ranged from 1 (healthy) to 5 (dead)

Interestingly, the S_2 progeny of T3-577, planted at the same time, had a mean root score of 2.3. This value is higher than that of the BC_1 progeny (Table 3). Similar results for T3-577 were observed at the South Coast Field Station (Table 4).

Crosses with 527OHK

Crosses between individuals having similar levels of resistance, such as UC-T3 and 527OHK, were evaluated in

Table 1. Chi-square contingency table of expected values and probabilities for greenhouse-grown UC-T3 S_1 progeny. The rating scale for root scores ranged from 0 (healthy) to 7 (dead)

Model	Scores (0–3)	Scores (3–7)	Chi-square	Probability
Single dominant gene (3:1)				
Observed:	44.0	29.0	7.77	0.005**
Expected:	54.8	18.2		
Two dominant loci (9:7)				
Observed:	44.0	29.0	0.32	0.575 NS
Expected:	41.1	31.9		

NS = No significant difference
** Significant at the 1% level

Table 2. Mean root scores of 12 UC-T3 S_2 families ranked according to disease severity in two trials in Oxnard, CA (November, 1987). Scores ranged from 1 (healthy) to 5 (dead)

Family	Field A	$LSD_{0.05}$*	Family	Field B	$LSD_{0.05}$**
T3-568	1.5	a	T3-AG29	1.3	a
T3-AG40	1.6	a	T3-571	1.5	a
T3-AG29	1.6	a	T3-AG35	1.6	a b
T3-AG41	1.6	a	T3-AG38	1.6	a b
T3-571	1.6	a	T3-AG39	1.6	a b
T3-AG20	1.8	a	T3-AG20	1.8	a b c
T3-AG38	1.8	a	T3-AG36	2.0	a b c d
T3-AG39	1.8	a	T3-AG34	2.1	a b c d
T3-AG28	1.9	a	T3-568	2.3	a b c d
T3-AG35	2.0	a	T3-AG28	2.5	b c d
T3-AG36	2.0	a	T3-AG41	2.6	c d
T3-AG34	2.1	a	T3-AG40	2.7	d
527OR	3.4	b	527OR	4.0	e

* $LSD_{0.05} = 0.75$ ($r = 8$)
** $LSD_{0.05}$ = varies from 0.80 to 0.92 due to unequal sample size ($r = 6-8$)

Table 3. Progeny mean root scores (MRS) and standard deviations (SD) of selected UC-T3 S_1 individuals (T3-719, T3-AG38, and T3-579) crossed with 527OR. Plants of the tolerant variety, 527OHK, and the highly susceptible variety, 527OR, were included for comparison. This field trial was conducted in Salinas, CA (September, 1987). Disease ratings: 1 = healthy, 5 = dead

Cross	No. of progeny	MRS (SD)	$LSD_{0.01}$*
$527OR_1 \times$ T3-719	10	2.2 (0.8)	a
$527OR_2 \times$ T3-AG38	10	3.0 (0.0)	b
$527OR_3 \times$ T3-577	10	3.4 (0.5)	b
527OHK (control)	11	3.5 (0.5)	b
527OR (control)	10	4.1 (0.3)	c

* $LSD_{0.01} = 0.59$

Table 4. Mean root scores (MRS) and standard deviations (SD) of backcross progeny (T3-577 × 527OR) and the parental lines evaluated at the South Coast Field Station (February, 1987). The analysis of variance showed significant differences among the progeny means at the 1% level ($P < 0.001$). Disease rating: 1 = healthy, 7 = dead

Cross	No. of progeny	MRS (SD)	$LSD_{0.05}$*	$LSD_{0.01}$**
T3-577	21	1.38 (0.67)	a	a
527OR × T3-577	26	2.00 (0.72)	b	a
527OR (seed)	20	3.30 (1.03)	c	b

* $LSD_{0.05}$ – Due to unequal sample size, LSD values ranged from 0.46 to 0.49
** $LSD_{0.01}$ – Due to unequal sample size, LSD values ranged from 0.64 to 0.68

Table 5. Mean root scores (MRS) and standard deviations (SD) of progeny of four crosses with UC-T3 selection and 527OHK evaluated in Oxnard, CA (February, 1987). Selected S_1 progeny of T3 were crossed with S_1 progeny of a cell culture line initiated from 527OHK. MRS of progeny obtained by self-pollinating the parents are included for comparison. Commercial seed of 527OR and 527OHK had MRS of 3.4 and 2.0, respectively. Disease ratings: 0 = healthy, 7 = dead

Cross	Genotype	No. of plants	MRS (SD)	P*	$LSD_{0.05}$**
1	T3-577 × HK15-569	27	0.9 (0.8)	0.0497	a
	T3-577 (selfed)	14	1.6 (0.9)		b
	HK15-569 (selfed)	10	1.6 (0.8)		b
2	HK15-572 × T3-575	13	1.2 (0.9)	0.002	a
	HK15-572 (selfed)	10	1.5 (0.7)		a
	T3-575 (selfed)	13	2.2 (0.4)		b
3	HK-572 (selfed)	10	1.5 (0.7)	>1	a
	HK-572 × T3-577	15	1.5 (0.7)		a
	T3-577 × HK15-572	11	1.6 (1.1)		a
	T3-577 (selfed)	14	1.6 (0.9)		a
4	HK15-574 × T3-575	12	1.1 (0.9)	0.001	a
	HK15-574 (selfed)	15	1.7 (0.8)		a b
	T3-575 (selfed)	13	2.2 (0.4)		b
	T3-575 × HK15-574	13	2.3 (1.0)		c

* P – Probability obtained from the analysis of variance
** $LSD_{0.05}$ – due to unequal sample size, LSD values ranged from 0.54 to 0.68 for cross 1, from 0.56 to 0.61 for cross 2, and from 0.62 to 0.64 for cross 4

Oxnard (Table 5). There was no consistent pattern of response. In one instance, cross no. 1 for example, the progeny resulting from the cross were significantly healthier than the selfed progeny of either parent, yet there were no significant differences between the progeny of crosses and the progeny obtained by selfing the parents in cross no. 3. However, in two crosses, HK15-572 × T3-575 (no. 2) and HK15-574 × T3-575 (no. 4), the progeny of the crosses showed disease symptoms comparable to the more resistant parent. Cross no. 4 was also interesting because the reciprocal crosses were significantly different.

Discussion

A report on the inheritance of FOA_2 resistance in crosses between celery and a highly resistant relative of celery, celeriac line 112 (*Apium graveolens* L. var rapaceum), suggested that resistance was governed by a single dominant gene with modifiers (Orton et al. 1984). A recent study of the F_2 progeny of crosses between 112 and the moderately resistant 527OHK, and the highly susceptible 527OR showed that at least two loci are involved (Quiros 1987). These results are consistent with those obtained with progeny of UC-T3; two independently segregating loci appear to be responsible for resistance to FOA_2 (Table 1). No additive or synergistic effects were noted; progeny of crosses between UC-T3 and other lines with moderate resistance to FOA_2 were generally equal to the most tolerant parent.

From this information, we propose that the original 527OR plant we cultured possessed the genotype aabb. Our first regenerated plant, plant T (Heath-Pagliuso et al. 1988), which received a rating of 2 on a scale of 0–7, most likely had the genotype aaBb or aAbb. After a second cell culture cycle the regenerant (UC-T3), which received a 0 rating when scored at 10 weeks, probably contained a second mutation making its genotype AaBb. Upon self-pollinating such an individual, the two loci would segregate, typically resulting in a broad phenotypic distribution, which is what was observed in the S_1 progeny of UC-T3 (Fig. 1).

We have shown that although the progeny of UC-T3 appeared to be segregating for two loci, the average resistance among the S_1 families or BC_1 progeny arrays was always significantly greater than that found in 527OR. In addition, excellent levels of resistance were obtained for many of the S_1 families (S_2 progeny) and among the progeny of crosses between UC-T3 S_1 individuals and the moderately tolerant variety, 527OHK. We conclude, therefore, that somaclonal variation for FOA_2 resistance is heritable and that the new germplasm could be incorporated into a breeding line. The progeny of UC-T3 have been well characterized for disease resistance (Heath-Pagliuso et al. 1988; Rappaport and Heath-Pagliuso 1986, 1987) and for horticultural traits (Rappaport and Heath-Pagliuso 1987), making our breeding line (Heath-Pagliuso et al. 1989) a valuable source of FOA_2-resistant germplasm.

Acknowledgements. We thank R. Brendler and A. Greathead, Farm Advisors, respectively, in Oxnard and Monterey Counties, and R. Klein, Superintendent, South Coast Field Station, Or-

ange County, for their assistance with field plots. Growers in Arroyo Grande, Salinas, Ventura, Oxnard, and Orange County are also acknowledged for their assistance and generosity in providing land for field plots. We express appreciation to R. A. Thompson and Sue Seely for technical assistance. We also acknowledge generous financial support from the California Celery Research Advisory Board, the Binational Agricultural Development Board (BARD), and the College of Agriculture, University of California, Davis.

References

Al-Abta S, Collin HA (1978) Control of embryoid development in tissue cultures of celery. Ann Bot 42:773–782

Charudattan R, DeVay JE (1972) Common antigens among varieties of *Gossypium hirsutum* and isolates of *Fusarium* and *Verticillium* species. Phytopathology 62:230–234

Chen CH (1976) Vegetative propagation of the celery plant by tissue culture. Proc SD Acad Sci 55:44–48

Daub M (1986) Tissue culture and the selection of resistance to pathogens. Annu Rev Phytopathol 24:159–186

Dunstan DI, Short KC, Merrick ME, Collin HA (1982) Origin and early growth of celery embryoids. New Phytol 91:121–128

Earle ED, Gracen VE, Yoder OC, Germmill KP (1978) Cytoplasm-specific effects of *Helminthosporium maydis* race T toxin on survival of corn mesophyll protoplasts. Plant Physiol 61:420–424

Evans DA, Sharp WR (1983) Single gene mutations in tomato plants regenerated from tissue culture. Science 221:949–951

Evans DA, Sharp WR, Medina,Filho HP (1984) Somaclonal and gametoclonal variation. Am J Bot 71:759–774

Heath-Pagliuso S, Pullman J, Rappaport L (1988) Somaclonal variation in celery: screening for resistance to *Fusarium oxysporum* f. sp. *apii*. Theor Appl Genet 75:446–451

Heath-Pagliuso S, Pullman J, Rappaport L (1989) 'UC-T3 somaclone': celery germplasm resistant to *Fusarium oxysporum* f. sp. *apii*, race 2. Hort Science 24:711–712

Larkin PJ, Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Larkin PJ, Ryan SA, Brettell RIS, Scowcroft WR (1984) Heritable somaclonal variation in wheat. Theor Appl Genet 67:443–455

Lee M, Phillips RL (1988) The chromosomal basis of somaclonal variation. Annu Rev Plant Physiol, Plant Mol Biol 39:413–437

McNay JW, Chourey PS, Pring DR (1984) Molecular analysis of genomic stability of mitochondrial DNA in tissue-cultured cells of maize. Theor Appl Genet 67:433–437

Nadel BL, Altman A, Ziv M (1989) Regulation of somatic embryogenesis in celery cell suspensions. I. Promoting effects of mannitol on somatic embryo development. Plant Cell Tiss Org Cult 18:181–189

Orton TJ (1984) Genetic variation in somatic tissues: method or madness? Adv Plant Pathol 2:153–189

Orton TJ, Durgan ME, Hulbert SH (1984) Studies on the inheritance of resistance to *Fusarium oxysporum* f. sp. *apii* in celery. Plant Dis 68:574–578

Pullman GS, Rappaport L (1983) Tissue culture induced variation in celery for *Fusarium* yellows. Phytopathology (Abstr) 73:818

Quiros CF (1987) Breeding celery for disease resistance and improved quality. In: Annual report, California Celery Growers Assn, Dinuba/CA, pp 19–22

Rappaport L (1982) Cloning celery for plant propagation. In: Annual report, California Celery Growers Assn, Dinuba/CA, pp 25–36

Rappaport L, Heath-Pagliuso S (1986) Cloning celery for plant propagation. In: Annual report, California Celery Growers Assn, Dinuba/CA, pp 6–11

Rappaport L, Heath-Pagliuso S (1987) Cloning celery for plant propagation. In: Annual report, California Celery Growers Assn, Dinuba/CA, pp 25–31

Rappaport L, Heath-Pagliuso S, Pullman G, Matlin S, Fuji D (1988) Somaclonal variation as a genetic source of resistance to *Fusarium* wilt of celery: a case history. In: Application of Biotechnology in Agriculture. Li Xu Hsien ed. Shanghai Scientific and Technical Publishers, Shanghai, P.R.C. pp 137–154

Schneider RW (1984) Effects of nonpathogenic strains of *Fusarium oxysporum* on celery root infection by *F. oxysporum* f. sp. *apii* and a novel use of the Lineweaver-Burk double reciprocal plot technique. Phytopathology 74:646–653

Scowcroft WR, Larkin PJ, Brettell RIS (1983) Genetic variation from tissue culture. In: Helgeson JP, Deverall BJ (eds) Use of tissue culture and protoplasts in plant pathology. Academic Press, New York, pp 139–162

Shepard JF (1981) Protoplasts as sources of disease resistance in plants. Annu Rev Phytopathol 19:145–166

Singer SR, McDaniel CN (1984) Selection of amitrole-tolerant tobacco calli and the expression of this tolerance in regenerated plants and progeny. Theor Appl Genet 67:427–432

Steel RGD, Torrie JH (1960) Principles and procedures of statistics. McGraw-Hill, New York pp 72–75 and 113–114

Williams L, Collin HA (1976) Growth and cytology of celery plants derived from tissue cultures. Ann Bot 40:333–338

Wright JC, Lacy ML (1988) Increase of disease resistance in celery cultivars by regeneration of whole plants from cell suspension cultures. Plant Dis 72:256–259

Zong-xiu S, Cheng-Zhang Z, Kang-le Z, Xiu-fang Q, Ya-Ping F (1983) Somaclonal genetics of rice *Oryza sativa*. Theor Appl Genet 17:67–73