Occurrence of Off-type Plants in japonica/indica Hybrid Rice Cultivars

Jeom-Ho Lee*, Yong-Hee Jeon, and Hung-Goo Hwang
National Crop Experiment Station, Suwon, 441-744, Korea

ABSTRACT

Frequent occurrence of off-type plants in a given cultivar has been a serious problem in both breeder's and farmer's fields. An experiment was designed to examine the differences in rate of occurrence of off-type plants among Tongil-type cultivars (high yielding cultivars derived from indica/japonica hybridization) from which the possible cause of higher occurrence of off-type plant in a specific cultivar was deduced. Among five Tongil-type cultivars examined for morphological variant in the field, only one cultivar, Dasanbyeo, had off-type plants. When analyzed with SSR markers, off-type plants showed different band patterns from original cultivar, having several extra bands in addition to cultivar-specific band, suggesting that off-type plants were originated from Dasanbyeo, rather than originated from mixing or mishandling of seed materials with other cultivars. The possible cause of off-type occurrence seems to be natural pollination with other cultivars adjacent to the original cultivar during seed multiplication. This was supported from the observation that self-crossed progeny of the off type plants showed a wide range of variation of agronomic traits which could not be observed when there was a smaller introduction of genes to the fixed germplasm as happened in the case of cultivar mutation. Another evidence supported this idea that Dasanbyeo showed much of difference in floral organ and behavior to other cultivar to be subjected to higher out-crossing than other cultivars examined.

Key words **:** rice, off-type, DNA marker, out-crossing

INTRODUCTION

Low yields and poor growth often stem from seed impurity of rice in farmers' field. The problem of seed impurity may be arose from mixing of other seed lots and/or natural mutation and out-crossing in the filed condition (Tsai, 1976; 1986; 1987; 1989; 2001; Ishikawa *et al.*, 1991; Charles *et al*, 2001). It had not been serious problem in Korea before the direct-seeding was introduced where transplanting with irrigation would eliminate the weedy and red rice during their germination (Lee *et al.*, 1990; Song *et al.*, 1992). This problem also arose in the other countries such as United State and South American countries in which the direct-seeding in rice is favored (Diarra *et al.*, 1985; Smith, 1989; Catala, 1995).

Besides the seed impurity problems in farmers' field, frequent occurrence of off-type plants in breeder's

*Corresponding author : **Jeom-Ho Lee,** E-mail : ljh@rda.go.kr

fields are not big concerns for the yield. Rather, the genetic contamination by introduction of foreign source would deteriorate the further improvement of genetic improvement and occurrence of the off-type in genetically modified crop plants (Whalon & Moris, 1996; Lemaux, 1999). The off-type incidence in breeder's field could result from intra- or inter-specific out-crossing with other genotype of the same crop species (Xu & Li, 1988; Song, 2003). Rice is a self-pollinated species with out-crossing rates that are usually <1%; however, higher out-crossing rates have been reported (Hoshikawa, 1989). Other cereal crops showed a similar tendency like rice. For example, wheat was reported with out-crossing rates less than 1% with variation from 0.3 to 6.1% depending on cultivars. High out-crossing rates are of little concern in large commercial fields because the majority of out-crossing will be intra-varietal. In contrast, problems may arise in the case of small plot increases where several genotypes are grown in close proximity. High out-crossing rates can result in obvious off-types if morphologically diverse cultivars are grown in adjacent pedigreed seed plots (Hulc & Matus-Cadiz, 2001).

Another reason for this off-type incidence in breeder's field may be a very small range of minor segregation in unfixed traits in advanced generation. The third reason maybe arose of natural mutation in filed condition.

Although the frequent occurrence of the off-type plants in breeders fields are reported, the limited knowledge of cause of the incidence made the objective of this study to examine differences in rate of occurrence of off-type plants among japonica/indica hybrid cultivars (Tongil-type cultivars). And the possible cause of higher occurrence in a specific cultivar was deduced from the observation.

MATERIALS AND METHODS

Plant materials used in this experiment were Tongil-type cultivars which was developed from crosses between indica and japonica cultivars. Five cutivars, Dasanbyeo, Andabyeo, Namcheonbyeo, Suweon 470, and Suweon 476 were seeded on April 23 and one-month-old seedlings were transplanted in a paddy field with a planting density of 220,000 plants /ha. Other cultural practices such as application of fertilizers were followed by Guidelines of Agricultural Experiment and Data Management (Rural Development Administration, 1983).

Each of 1,350 individual plants in a given cultivar was screened by morphological and physiological characteristics such as plant maturity to investigate occurrence of off-type plants in field conditions. The observed off-type plants and their original cultivar were subjected to further analysis of genetic variation through DNA fingerprinting analysis. DNA analysis was done with cultivar-specific microsatellite markers; in this experiments, six Dasan-specific primers, RM048, RM204, RM249, RM257, RM148, and RM070 (Kwon *et al.*, 2001). PCR amplification was done by method described by Song *et al.*(2002).

To analyze the phenotypic variation, off-type plants were self-crossed and their F_2 plants were subjected being investigated for phenotypic variation range. Each of twenty-five F_2 progenies from each self-crossed off-type plant were observed for heading date, culm length, panicle length, and panicle number in a plant.

The comparison of floral organ morphologies and behavior between Dasanbyeo and Namcheonbyeo was conducted in a greenhouse condition. The difference in duration of pistil opening, flowering angle, stigma length, and pollen acceptability of pistil which was estimated by seed-set duration after the day of emasculation (Takeda, 1991; Paul, 1994).

RESULTS AND DISCUSSION

Occurrence of off-type plants and their agronomic characters

Each of 1,350 individual plants in Dasanbyeo, Andabyeo, Namcheonbyeo, Suweon 470, and Suweon 476 were screened by morphological and physiological characteristics in field condition. A very clear cultivar difference was observed in occurrence of off-type plants; among six cultivars examined, only one cultivar, Dasanbyeo showed occurrence of off-type plants with the rate of 0.06 % (Table 1). The phenotypic appearance of most off-type is inferior to original cultivars (Table 2). Late heading, tall plant status, more useless tillers and panicle length, lighter grain weight, and lower fertility were observed in most of off-type plants. The severest one is the lower levels of grain fertility which is often found in progenies of genetically-distant crossing combination such as indica and japonica hybrid breeding (Xu & Li, 1988).

Table 1. Cultivar difference in off-type occurrence of Tongil-type cultivars

Cultivars	Plant No. Examined	Off-type plants	
		No.	%
Dasanbyeo	1,350	8	0.6
Andabyeo	1,350	0	0
Namcheonbyeo	1,350	0	0
Suweon470	1,350	0	0
Suweon476	1,350	0	0

The varietal difference in the occurrence of off-type is reported in the other cereal crops. For example, wheat

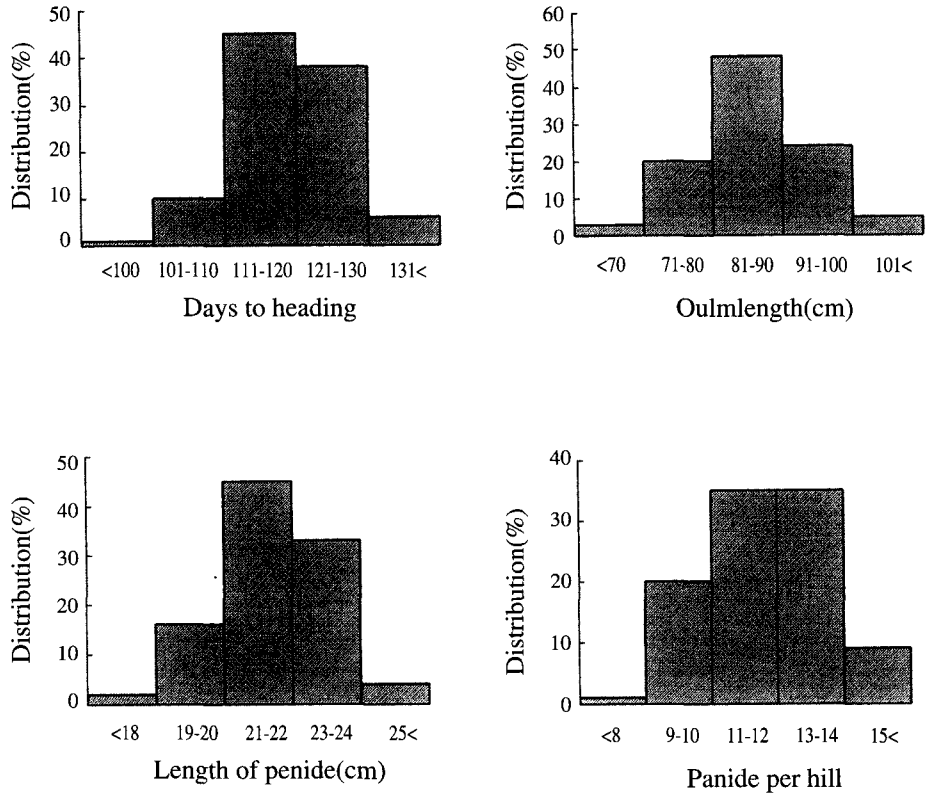

Fig. 1. Distribution of agronomic trait of selfed progenies of off-type rice.

Table 2. Comparison of agronomic characteristics between Dasanbyeo and off-type plants

Designation	Days to heading(day)	Culm length(cm)	Panicle length(cm)	Spikelets /panicle(no.)	Filled grain(%)	1,000 grain weight(g)
Dasanbyeo	112°æ0.8a	78°æ1.4	24°æ0.6	121°æ1.9	84°æ1.5	24°æ0.4
Off-type plants	121°æ1.4	99°æ4.0	26°æ1.4	129°æ6.1	17°æ8.2	21°æ1.1
Difference**	**	Ns	*	**	*	

†; Mean ± 2 S.E.

‡ ns : not significant, *, ** : significant at 5% and 1% level, respectively

is a self-pollinated species with off-type incidence less than 1% as higher rates have been reported (Hucl & Matus, 2001). They all assume this is from the out-crossing. Other factors influencing off-type incidence is pollen dispersal also contributes to the appearance of off-types in adjacent pedigreed seed plots (Hulc & Matus, 2001). They concluded that high out-crossing rates and optimum conditions for pollen dispersal may lead to an unacceptable level of off-types in the production of pedigreed seed.

The results from this experiment indicates that the varietal difference in the off-type incidence is from the genetic factor rather than outside factor such as climatic condition and plot spacing and location and, handling of seed plots.

DNA analysis of off-type plants and original cultivar

To see the origin of the off-type plants, Dasanbyeo and off-type plants found in Dasan plot were subjected to DNA fingerprinting with Dasanbyeo-specific microsatellite markers, RM048, RM204, RM249, RM257, RM048, and RM070 (Fig. 1). These primers already known to produce one specific allele inherent to Dasanbyeo in previous experiment with several hundred Korean rice germplasm (Kwon *et al.*, 2001). The left lane in each gel is amplified products of Dasanbyeo and others are off-type individuals. Most of the primers produced 1 bands in Dasanbyeo, specific bands for Dasanbyeo, while off-type plants produced multiple bands including the Dasanbyeo-specific bands.

These results indicated that the off-type plants examined was originated from Dasanbyeo, whether by the way of natural mutation or out-crossing with other cultivar plot adjacent to Dasanbyeo plot, and evidence of which is demonstrated with the appearance of Dasanbyeo-specific band in off-type plants.

Segregation patterns of traits in progenies of self-crossed off-type plants

The hypothesis that this occurrence is not from the single mutation or minor segregation, there will be a wide ranges of segregation in F_2 plant will be conducted by progeny segregation test. To do this, off-type plants were self-crossed and their F_2 plants were subjected being investigated for phenotypic variation range.

As expected, there are a wide ranges and continuous variation in examined traits. Hading date in off-type showed a mean of late heading than Dasanbyeo with wide variation of from <100 days to >130 days. Culm length, panicle length, and number of panicle showed

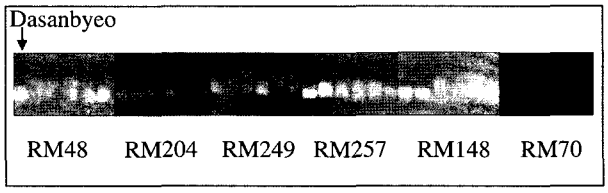

Fig 2. DNA analysis of Dasanbyeo and the off-type plants by microsatellite makers. The left lane of each primer panel is Dasanbyeo, and other fives are off-type plants

Table 3. Comparison of floral organ and inflorescence behavior between cultivars

Cultivars	Flowering duration(min.)	Flowering Angle(°)	Stigma length(mm)	Pollen acceptability† (days)
Dasanbyeo	72	32	5	7
Namcheonbyeo	52	25	3	5

†Pollen acceptiblity of pistil was measured by seed-set duration after the day of emasculation.

the same tendency as heading, as mean difference from Dasanbyeo with wide range of variation. If off-type plants resulted from a single mutation or minor segregation of advanced generation (for example F_2 in Dasanbyeo), theoretical and empirical data could not support this wide variation in the F_2 population.

Examination of floral organ of off-type plants

From the data examined before, the possible cause of the off-type plants was the increased out-crossing in Dasanbyeo. We examined why this increase occurred in Dasanbyeo, compared to other cultivars, comparison of floral organ morphologies and behavior between Dasanbyeo and Namcheonbyeo was conducted in a greenhouse condition. The difference in duration of pistil opening, flowering angle, stigma length, and pollen acceptability of pistil between two cultivars was observed (Table 3).

The more favorable condition for out-crossing was found in Dasanbyeo than Namcheonbyeo. For example, Dasanbyeo has more prolonged flowering opening duration (72 min.) than Namchem (52 min.) which accommodate more chances for the pollens from other plants to stick the stigma. Also, more opening by widened angle (32 degree in Dasanbyeo versus 25 degree in Namcheonbyeo), longer stigma and more prolonged seed-set duration (expressed as pollen acceptability of pistil) seems to be more out-crossing would happen in Dasanbyeo.

Concluding remarks

The results obtained from present study can be summarized as follows; 1) There is a varietal difference in off-type plant incidence, 2) the off-type plants are originated from the original plants 3) The cause of the off-type incidence maybe from the increased out-crossing in specific cultivar, and 4) The increased out-crossing is closely related with floral organ and their inflorescence behavior.

Although this research is focused on the occurrence of the off-type to natural mutation in several location or genes with greater interaction to other physiological and phenotypic variation would not be completely escaped. More accumulated knowledge of this area will be needed for further and confirmed results in future.

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