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Potato Breeding for Combined Resistance to Tropical Pests by the Ploidy Level Manipulation Approach: Progress in Extraction of Haploids with Male Fertility and 2n Pollen

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ABSTRACT

The present approach is aimed at combining multiple resistance to important pests of tropical zones working at the 2x level. This permits an efficient breeding work using disomic inheritance. Three steps are involved: (1) extraction of haploids from selected tetraploids; (2) population breeding at the 2x level for multiple resistance, agronomic characteristics, adaptation and 2n-pollen production in wide genetic backgrounds; and (3) transfer of the improved 2x germplasm into the 4x level via First Division Restitution (FDR) 2n-pollen.

The following progress has been attained for the first step. A large number of haploids were extracted from selected tetraploids by 4x - 2x crosses using the diploid Phureja clone lvP 35 as a haploid inducer. More than 400 haploids coming from late blight-resistant tetraploids were evaluated for resistance in a greenhouse. Cytological finding of male fertility and 2n-pollen production in these haploids is encouraging, because the male fertility of the haploids is useful for crossing with other valuable diploids, and the 2n-pollen can be utilized to transfer the resistance of the haploids into 4x progeny through 4x - 2x crosses. Many PVX/PVY-resistant haploids were extracted from resistant tetraploids, and 45 haploids were selected for good agronomic characters. A total of 242 haploids obtained from cyst-nematode-resistant Andigena clones is being evaluated for resistance. Finally, further extraction of haploids from tetraploids with resistance to PVX/PVY, PLRV, cyst nematode, late blight, or heat is being done.

Introduction

The control of pest and disease by resistant cultivars of potato is important since the average farmer in developing countries has limited access to chemical control. Moreover, use of certified seed is limited in some countries and is not available in others. These facts, and the greater severity of pest and disease in the tropics, justify a strong emphasis on breeding for multiple pest and disease resistance.

The great majority of cultivated potatoes are autotetraploids ( $2n = 4x = 48$ ) and have tetrasomic inheritance. Tetrasomic inheritance is a disadvantage for combining resistances, especially when resistances are polygenically controlled.

The present approach aims to combine multiple resistance to important pests of tropical zones working at the 2x level. This permits efficient breeding work using disomic inheritance (Hougas and Peloquin, 1958). Three steps are involved: (1) extraction of haploids from selected tetraploids; (2) population breeding at the 2x level for multiple resistance, agronomic characteristics, adaptation, and 2n pollen production in wide genetic backgrounds; the population breeding approach applies recurrent selection which increases desirable gene frequencies and stimulates their recombination so that a higher frequency of multiple resistant progenies can be obtained; and (3) transfer of the improved 2x germ plasm into the 4x level via First Division Restitution (FDR) 2n pollen.

Three conditions should be satisfied before full potential of the approach using haploids and 2n pollen can be exploited. First, a large number of haploids with wide genetic background should be obtained since intensive use of particular haploids results in narrowing the genetic bases. This might lead to coancestry and inbreeding which is highly undesirable, particularly for yield. Second, a good number of male fertile haploids should be utilized so that male sterility caused by interactions between tuberosum cytoplasm and nuclear genes of related germ plasm can be avoided. This is important to achieve efficient crossing work during the advancing generations of population improvement. Finally, identification of the parallel spindle (ps) gene in haploids extracted from tetraploids is crucial, since the use of FDR 2n pollen by ps is essential to transfer improved 2x germ plasm into the 4x level. This paper reports progress of production of a large number of male fertile haploids with FRD 2n pollen production.

#### Materials and Methods

Two different ecological zones within the tropics are considered. These are highland tropics and lowland tropics. The resistances or tolerances considered important for each zone are:

- highland tropics - PVX, PVY, cyst nematode, late blight and frost.
- lowland tropics - PVX, PVY, PLRV, bacterial wilt, root-knot nematode, late blight, potato tuber moth and heat.

Selected tetraploids were used as sources of resistance to PVX, PVY, PLRV, cyst nematode, late blight, frost and heat and, thus, haploid extraction from the selected tetraploid was needed.

Extraction of haploids from selected tetraploids was done by 4x - 2x crosses by using a diploid clone IvP 35, which was reported to be a good haploid inducer with embryo-spot marker (Hermsen and Verdenius, 1973). Seeds from the crosses were checked for embryo spot. Observation of general morphology and count of chloroplast number of stomata were used to eliminate triploid or tetraploid plants grown from embryo spotless seeds.

Pollen stainability and 2n pollen production were studied in haploids from which pollen samples were available. The mechanism of 2n pollen production was determined by cytological observation of meiosis in floral buds.

## Results and Discussion

### Extraction of large number of haploids

A sample of 2,379 haploids with different attributes and genetic background was obtained from 76 tetraploids (Table 1). Extraction of a large number of haploids from late blight resistant tetraploids was emphasized, since only a small proportion of the haploids were expected to be highly resistant. Since the haploids were from many tetraploids having tuberosum, andigena and phureja in their genetic background, it is expected that use of the haploids will not lead to inbreeding unless the number of useful clones becomes very small making mating between relatives difficult to avoid. The haploids are being evaluated for resistance.

Table 1. Numbers of haploids extracted from selected tetraploid.

Resistance of tetraploid parent	Number of resistant parents	Genetic background	Number of extracted haploids
Late blight	21	tbr, adg	1,726
PLRV	10	tbr, adg	180
PVX/PVY	16	tbr, adg	210
Cyst nematode	17	adg	118
Heat	12	tbr, adg, phu	145
	<u>76</u>		<u>2,379</u>

The haploid inducer IvP 35 was relatively efficient in terms of number of haploids obtained after pollination, although the number of haploids per pollination varied much depending on the female parents. Thus, it would be advisable to test many cultivars as a source of haploids and then concentrate haploid extraction work on some selected tetraploids based on their ability to produce many haploids. Some portion of seedlings from spotless embryo seeds turned out to be triploids or tetraploids. This was probably due to incomplete expression of embryo-spot marker genes and/or involvement of modifying genes of female parents which inhibit the expression of the genes.

### Male fertility of the extracted haploids

A sample of 501 haploids has been studied for pollen stainability. The haploids were classified into four groups according to pollen stainability. About a third of the haploids had less than 5% of pollen stainability and were considered to be male sterile and 29% of the haploids had 5% to 30% of pollen stainability and were expected to have only low level of male fertility (Table 2). On the other hand, 14% of the haploids had 30% to 50% of stainability and were expected to have good level of male fertility and the rest, 22% of the haploids, had more than 50% of the stainability and were considered highly male fertile. Thus, 36% of the haploids are expected to have an adequate level of pollen fertility. Since pollen stainability is an indirect estimate of pollen fertility, real fertility value should be obtained from the amount of seed produced in 2x - 2x crosses. Some of the haploids with more than 30% of stainability were used for 2x - 2x crosses as male parents and high seed sets were obtained. The results are

encouraging, since use of haploids as male parents makes it possible to circumvent cytoplasmic-genic male sterility in hybrids with other 2x germ plasm and to keep good level of male fertility during population improvement at the 2x level.

Table 2. Distribution of pollen stainability values in extracted haploids.

Pollen stainability (%)	<5	5-30	30-50	>50	Total
Number of haploids	179 (36%)	144 (29%)	70 (14%)	108 (22%)	501

Several genetic factors are supposedly involved in determining level of male fertility of extracted haploids. They are: (1) cytoplasmic factors (Koopmans, 1959; Grun et al., 1962); (2) gene(s) which interact with sensitive cytoplasm causing cytoplasmic-genic male sterility (Grun et al., 1966, 1979); (3) restorer gene(s) which restore male fertility in plants which otherwise have cytoplasmic-genic male sterility (Hanneman and Peloquin, 1979; Peloquin and Iwanaga, 1981); (4) male sterile gene(s); (5) minor gene(s) which reduce level of male fertility.

Male sterility is common in haploids extracted from North American or European varieties (Ross et al., 1964). Carroll and Low (1976) reported that about 70% of their extracted haploids from four varieties had less than 10% of pollen stainability. The present study shows higher level of stainability in the haploids compared with those of Carroll and Low (1976). Use of many hybrids between tuberosum x andigena as a source of haploids was probably one of the reasons for the high stainability since chance of exposure of male sterile recessive genes is minimum from such heterozygous hybrids. Landeo and Hanneman (1979) attributed high level of male fertility of haploids from andigena clones to their heterozygosity. Another possibility is that many tuberosum parents utilized might have demissum cytoplasm, but not tuberosum cytoplasm which results in male sterility interacting with nuclear genes. Since most of the tuberosum parents resistant to late blight have demissum in their genetic background, identification of cytoplasm donor for the tuberosum parents is important to know to avoid accumulation of an increasing frequency of male sterility over several crossing and selection cycles. This is true, especially where modern day varieties and advanced clones stem from interspecific crosses, e.g. involving demissum, acaule, stoloniferum, chacoense, vernei. If the hypothesis is true, late blight resistant tetraploids which have demissum cytoplasm would be an excellent source of male fertile haploids.

#### Identification of FDR 2n pollen production in the extracted haploids

Screening work for 2n pollen production in 322 haploids with more than 5% of pollen stainability from 19 tetraploids resulted in finding 151 haploids with 2n pollen (Table 3). Thus, about half of the haploids were 2n pollen producers. Gene frequency of ps was estimated for the haploids from cv. Atzimba and for the haploids from other 18 tetraploids, assuming that ps was the only 2n-pollen production mechanism involved in the haploids. High ps frequencies were obtained in both populations, 0.71 and 0.77, respectively. This is in accordance with the high frequency of ps in tuberosum cultivars reported by Iwanaga and Peloquin (1982). Based on the segregation rate for 2n pollen production, genotype of

Atzimba for ps was determined as Ppspsps. The present results clearly show that ps gene is common in haploids and extracted haploids are a good source of ps gene.

Table 3. 2n pollen production of haploids extracted from Atzimba and 18 other tetraploid and estimated ps frequencies.

Tetraploid parents	No. of haploids studied	Without 2n pollen	With 2n pollen	<u>ps</u> frequency
Atzimba	213	106	107	0.71
18 tetraploids	109	45	64	0.77
	<u>322</u>	<u>151</u>	<u>171</u>	

Meiosis study of some Atzimba haploids unveiled that about half of the haploids had synaptic abnormalities which were due to one recessive gene sy4 (synaptic mutant No. 4). The existence of mainly univalents at Metaphase I strongly indicates inhibited chromosome pairing (detailed cytological study of mutant will be published elsewhere). Usually, synaptic abnormalities result in male sterility due to abnormal chromosome distribution. However, when the mutant is combined with ps gene, fertile FDR 2n pollen with inhibited genetic recombination can be produced. The genetic significance of this combination of the mutants is that it makes possible the transmission of 100% of heterozygosity and epistasis of the parent into the 4x offspring (Okwuagwu and Peloquin, 1981). Therefore, the finding of sy4 mutant along with ps in Atzimba haploids is extremely valuable for the present breeding approach, since desirable gene combinations bred at the 2x level can be transferred intact into the 4x offspring. The progenies can be used in a clonal selection program or can be evaluated for possible utilization as True Potato Seed (TPS) cultivars. Moreover, the combined mutants will be especially useful for utilization of TPS for commercial potato production. The TPS cultivars produced by using the mutants would have minimum segregation thanks to genetically uniform pollen and desirable multiple resistances which are uniformly transmitted by FDR 2n pollen.

It seems that synaptic mutants are relatively common in potatoes. Iwanaga and Peloquin (1979) reported sy1 and emphasized the importance of further search of meiotic mutants for potato breeding. Since then, three synaptic mutants, sy2 (Johnston et al., 1981), sy3 (Okwuagwu and Peloquin, 1981), and sy4 (present report) were found. Therefore, a careful search for useful meiotic mutant in different breeding materials might lead to the discovery of more mutants. Moreover, it is likely that such mutants, if any, will be found together with ps which is very common in haploids, permitting the use of the combination of ps and sy with relative ease.

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