Euphytica **66**: 217–223, 1993.

Cytoplasmic male sterility in quinoa

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Received 5 October 1992; accepted 27 January 1993

Key words: Chenopodium berlandieri, Chenopodium quinoa, crop-weed gene flow, cytoplasmic male sterility, partial restoration, quinoa

Summary

The quinoa cultivar 'Apelawa' carries both normal and male sterile cytoplasms. Plants with male sterile cytoplasm produce flowers characterized by the complete absence of anthers and prominent exsertion of stigmas. Intraspecific crosses between male sterile quinoa plants and normal male fertile pollen donors consistently produced male sterile offspring under greenhouse conditions. Interspecific hybridization between male sterile quinoa plants and the related weed species *Chenopodium berlandieri* resulted in offspring with partial restoration of male fertility. The male sterile cytoplasm found in 'Apelawa' has potential for use in the production of quinoa hybrids, although a more complete restorer system than that identified in *C. berlandieri* would be desirable.

Introduction

Quinoa (*Chenopodium quinoa* Willd.), a traditional Andean pseudocereal, is attracting increasing interest as a potential crop for cool, dryland areas at higher elevations in the western United States. The palatability and nutritional qualities of the grain have created a demand for it in the gourmet and health food markets and the potential for processing quinoa in different ways is being explored. Current demand for quinoa in the United States exceeds domestic supply (Johnson & Croissant, 1990). Some Bolivian and Chilean quinoas perform well in Colorado and Wyoming at elevations above 2,000 meters, but progress in developing new cultivars suited to the region is limited by the lack of a reliable and efficient method of hybridization. Controlled crosses of selected parent plants would produce segregating generations of progeny from which individuals with new and desirable combinations of characters might be obtained. In addition, field observations of plants which are hand-crossed or appear to be spontaneous hybrids suggest that there is considerable potential for exploiting heterosis (D.L. Johnson, unpublished).

Most quinoas are highly self-fertile with copious pollen production. The perfect flowers are very small (3 to 4 mm in diameter) and clustered together in large numbers to form the inflorescence. Hybridization techniques involving manual emasculation and pollen transfer are therefore extremely difficult. The development of male sterile quinoa lines to be used as maternal parents in hybrid production has been suggested as an alternative method (Wilson, 1980; Risi & Galwey, 1984). Well-characterized male sterile systems have already been used to create hybrids in many crop species, including maize, sorghum, sugar beet and onion (Kaul, 1988). Such a system may also prove valuable in quinoa.

There have been occasional reports of cytoplasmic male sterility in quinoa. Gandarillas (1969) described a male sterile line which produced all male

sterile offspring when crossed and backcrossed to five different male fertile pollen parents; he concluded that in this instance the male sterile character was under cytoplasmic control. Simmonds (1971) reported an erratically transmitted male sterility in quinoa of Bolivian origin grown in Scotland, and suggested a nuclear gene generating cytoplasmic sterility, influenced by environmental factors. There has been no subsequent confirmation of this possibility. Risi & Galwey (1984) described work carried out in Peru by Aguilar, who reported cytoplasmic male sterility in the line 'UNTA 292'. This line produced male sterile progeny in the F_1 and on two successive backcrosses to male fertile pollen parents, but fertility was apparently restored when the Bolivian cultivar 'Sajama' was used as the pollen donor. Bolivian researchers have identified cytoplasmic male sterility in several quinoa populations (Saravia, 1991) although some forms are variable in the extent to which the trait is expressed (A. Bonifacio, personal communication). Despite these potentially promising results, a reliable and fully characterized system of male sterility in quinoa has still to be reported, and male sterile plants have not yet been used for commercial hybrid production in this crop (Johnson & Ward, 1992; Galwey, 1989).

Materials and methods

Twenty-nine plants with poor seed set (fewer than 40 seeds per plant) were collected in August 1989 from a field population of the Bolivian quinoa cultivar 'Apelawa' grown in the San Luis Valley (37° N) in southern Colorado. Seed was removed from the dried panicles by hand and planted in a greenhouse at Colorado State University, Fort Collins in January 1990. All subsequent work was carried out under greenhouse conditions between January 1990 and September 1992. Greenhouse temperature was maintained at 25° C and plants were grown under natural daylength with no artificial illumination; in Fort Collins this gives a 10 hour photoperiod in January, increasing to 16 hours by mid-June. Plants were raised in groups of four per 25 cm pot in commercial potting compost supplemented by a commercial liquid fertilizer. This technique resulted in mature plants 70 to 80 cm tall which began flowering eight weeks after germination and produced harvestable seed at 14 weeks.

All plants were examined at flowering for the presence of anthers and the production of pollen. Plants in which anthers containing pollen grains were present in any flowers were classified as male fertile. Plants without anthers or with no pollen production were classified as male sterile. In the case of male sterile plants, 10 flowers were selected at random from different parts of the inflorescence of each plant and examined microscopically. The stage at which anther development aborted was recorded.

Male fertile plants were self-pollinated by enclosing the inflorescence in a waxed paper pollination bag during anthesis. Male sterile plants were crossed with male fertile pollen donors by enclosing both inflorescences in a single pollination bag for 7 to 10 days and shaking the bag each day to promote pollen transfer. Pollen donors were also selfed and 20 progeny from each donor plant were grown to ensure that plants used as paternal parents were not heterozygous for recessive genic male sterility.

Four progeny were raised from each of the 29 plants collected, producing 29 F_1 families of unknown paternity, but presumed to be comprised of at least half-sibs. All male fertile plants in this generation were selfed, and the F_2 offspring raised and examined as described above. Twenty-two male sterile plants in the F_1 generation were pollinated using either a male fertile 'Apelawa' pollen donor, or '407', a line of Chilean origin which has undergone several cycles of selection for cultivation in Colorado. Offspring from these crosses were raised in the greenhouse as described above and examined at flowering for the presence of anthers and pollen. Male sterile progeny from these F_1 crosses were backcrossed to the pollen parent lines. They were also used as maternal parents in a series of hybridizations with male fertile pollen donors from 14 different South American quinoa cultivars, and the offspring were examined as previously described.

Interspecific crosses were made between male sterile quinoa plants derived from this hybridization series and nine accessions of *Chenopodium berlandieri*, a common weed species of the western

United States. Seven of the *C. berlandieri* samples were collected from field sites in eastern Wyoming, and two from southern Colorado. The F_1 hybrid progeny were examined at flowering for the presence of anthers and pollen as previously described. In addition, pollen grain viability in male fertile F_1 plants was tested by taking pollen samples from five plants in each F_1 family, and staining with cotton blue in lactophenol. Viability was estimated for each plant as the percentage of completely stained grains in a count of 200, made under a compound microscope. Male fertile F_1 plants were self-pollinated and sixty F_2 offspring from each of three F_1 plants from different families were raised. The F_2 plants were examined at flowering for the presence of anthers, and pollen viability was estimated for each male fertile plant as described above.

Results

Twenty-five of the 29 half-sib families consisted entirely of male fertile plants, but viable seed was obtained from only eight of these families when selfed. The F_2 generation from these eight male fertile families consisted of 352 plants, all with normal hermaphrodite flowers and good seed set. The other 17 male fertile families exhibited apparently normal pollen production, but selfing the plants produced either no seed or seed which subsequently failed to germinate. The remaining four of the 29 half-sib families each consisted of a mixture of male sterile and male fertile plants. Total progeny numbers for these four families are given in Table 1.

It was observed in the case of the male fertile plants in these four families that the percentage of flowers containing anthers in an individual inflorescence varied from over 90 to less than 10, and that such flowers were always concentrated at the distal ends of the clusters. Seed set from selfing was extremely poor, indicating that despite the presence of anthers, these plants were not fully self-fertile. Anthers were completely absent throughout the inflorescence in all plants classified as male sterile. Microscopic examination of male sterile flowers revealed a ring of incomplete filaments surrounding the ovary, each filament with a threadlike extension replacing the anther at the distal end. Occasionally a double ring of such filaments was observed. On some male sterile plants the flowers lacked stamens entirely. Prominent exsertion of the stigmas on the male sterile plants was observed in all generations.

A total of 159 progeny was obtained from five of the 22 crosses using male sterile plants as maternal parents and either 'Apelawa' or '407' as pollen donors. Details of the 5 crosses which resulted in progeny are given in Table 2. All offspring from these crosses were male sterile, and closely resembled the maternal parents in possessing either incomplete filaments or no stamens at all. Backcrosses between two of these male sterile progeny and 'Apelawa' or '407' pollen donors produced a total of 153 offspring, all male sterile, as shown in Table 3. Hybridizations between male sterile progeny from the crosses shown in Table 2 and 14 different South American quinoa cultivars produced a total of 284 offspring, also all male sterile. Details of these hybridizations are given in Table 4.

Six of the interspecific crosses using *C. berlandieri* as the pollen parent produced F_1 generations consisting entirely of male fertile plants. Two of the remaining crosses produced both male fertile and

Table 1. Segregation in half-sib F_1 families derived from four field accessions of the quinoa cultivar 'Apelawa'

Family	Male fertile	Male sterile
AP12	2	2
AP14	1	3
AP18	4	10
AP30	4	8

Table 2. Segregation among progeny from five male sterile F_1 quinoa plants crossed with two male fertile quinoa lines

Female parent	Male parent	Progeny	
		Male fertile	Male sterile
AP18-1	Apelawa	0	18
AP18-5	407	0	39
AP30-7	407	0	39
AP30-9	407	0	26
AP30-12	407	0	37

Table 3. Segregation among progeny from two male sterile quinoa plants backcrossed to male fertile paternal lines

Female parent	Male parent	Progeny	
		Male fertile	Male sterile
(AP18–1× Apelawa)	407	0	75
(AP18-1× Apelawa)	Apelawa	0	78

male sterile F_1 offspring, while one cross generated only male sterile F_1 progeny. Details of these F_1 generations are given in Table 5. Floral structure of all the male sterile F_1 hybrid plants resembled that of the maternal parent with incomplete filaments or stamens completely absent. In the male fertile F_1 plants, up to 50% of the flowers were found to be without anthers; flowers with anthers were grouped at the distal ends of the clusters. This spatial distribution of the male fertile flowers within the inflorescence, and the glomerulate weedy morphology of the inflorescence itself, resembled the appearance of the male fertile plants in the four segregating half-sib F_1 families derived from the original 'Apelawa' field accessions. Pollen grain viability for the interspecific F_1 hybrids ranged from 14.0% to 63.6%, compared to 98.2% for pollen from a normal male fertile quinoa plant grown under the same greenhouse conditions. All male fertile F_1 interspecific hybrids, however, set seed when selfed.

Details of the three F_2 families raised from interspecific hybrid F_1 male fertile parents are given in Table 6. Three levels of male fertility were observed in this generation: completely male fertile, with anthers present in at least 90% of flowers and pollen viability exceeding 85%; partially male fertile, with anthers in 10% to 60% of flowers and pollen grain viability below 65%, and male sterile with anthers completely absent from all flowers. Although all the F_2 plants fell readily into one of these three groups, no segregation ratio was observed that was consistent for all three families.

Discussion

The consistent transmission of male sterility through the female parent to produce male sterile progeny indicates a cytoplasmically inherited trait, similar to that described by South American researchers. Genic male sterility, controlled by a single recessive nuclear allele which results in fully-formed but empty anthers, has also been reported in quinoa populations grown in Colorado (Ward, 1991). The cytoplasmic form described here, how-

Table 4. Segregation among progeny from crosses between male sterile quinoa plants and South American quinoa cultivars

Female parent	Male parent	Progeny	
		Male fertile	Male sterile
(AP30-9× Apelawa)	Marangani (Peru)	0	41
(AP30-7× Apelawa)	Calcha (Bolivia)	0	10
(AP18-1× Apelawa)	Sajama (Bolivia)	0	37
(AP30-7× Apelawa)	Killu (Bolivia)	0	18
(AP18-1× Apelawa)	Kaslala (Bolivia)	0	18
(AP30-7× Apelawa)	Isluga (Bolivia)	0	19
(AP30-12× Apelawa)	Chuppi (Bolivia)	0	17
(AP30-12× Apelawa)	Baer (Chile)	0	21
(AP30-7× Apelawa)	Cahuil (Chile)	0	20
(AP30-7× Apelawa)	Chullpe (Chile)	0	19
(AP30-9× Apelawa)	Janco (Chile)	0	6
(AP30-7× Apelawa)	Kanchi (Chile)	0	11
(AP18-5× Apelawa)	Lihio (Chile)	0	20
(AP18-1× Apelawa)	Tango (Chile)	0	27

ever, is distinct both in mode of transmission and in morphological effects. The flowers which were observed with prominently exserted stigmas and vestigial or absent stamens resemble those described by Gandarillas (1969), but without further information on Gandarillas' material it is not possible to determine whether the quinoa cultivar 'Apelawa' carries the same male sterile cytoplasm. The cytoplasmic male sterility identified by Aguilar interacts with a dominant nuclear restorer factor present in the Bolivian quinoa cultivar 'Sajama' (Risi & Galwey, 1984). Using 'Sajama' as the pollen parent has no effect on the expression of male sterility in quinoa carrying the 'Apelawa' male sterile cytoplasm (Table 4), which indicates that this is a different cytoplasm from that found by Aguilar. The partial restoration of male fertility in the F_1 generation when *C. berlandieri* is used as the pollen parent with male sterile quinoa plants resembles an earlier observation made by Wilson (1980). This author carried out artificial hybridization between quinoa and *C. berlandieri,* using as maternal parent the cytoplasmic male sterile quinoa described by Simmonds (1971), and reported some of the offspring as possessing anthers, although he did not assess the extent of fertility restoration in the F_1 or obtain an F_2 generation. Simmonds' quinoa, like 'Apelawa', was of Bolivian origin, but the male sterility he described did not have the stability of expression and consistency of transmission observed in the 'Apelawa' male sterile cytoplasm. Unfortunately, Simmonds' material has since been lost (H. Wilson, personal communication). Given the apparent inconsistencies between these earlier reports, we would suggest that the considerable amount of genetic variation present in cultivated quinoa throughout its range in the Andes may well be reflected in the existence of several distinct forms of male sterility, both cytoplasmic and nuclear.

The reappearance of some degree of male fertility in hybrid F_1 plants from interspecific crosses between quinoa and *C. berlandieri* indicates that this related weed species carries a partially dominant restorer. If the restorer is a single gene, it is likely that the two weed accessions which produced mixed F_1 generations were heterozygous at the locus in question, while the restorative allele was not carried by CBSLV-2, which produced only male sterile F_1 offspring. The segregation of the F_2 generation into three distinct levels of male fertility suggests that full restoration requires homozygosity for at least one restorer allele, while heterozygosity results in only partial restoration, characterized by fewer flowers with anthers and reduced pollen viability. This was also seen in the hybrid F_1 generation. The lack of any consistent Mendelian ratio among the segregating F_2 families, and the range of fertility observed in the progeny classified as partially male fertile, suggest that this is not a simple one-gene restorer system. Additional modifier genes are almost certainly involved, and fertility in the hybrid progeny may also be reduced by incompatibilities be-

Table 5. Segregation among F_1 progeny from crosses between male sterile quinoa plants and nine accessions of *Chenopodium berlandieri*

Female parent	Male parent	Progeny		Pollen grain viability*
		Male fertile	Male sterile	
(Apelawa× Marangani)	CBWYO-1 (Wyoming)	20	0	14.0%
(Apelawa× Marangani)	CBWYO-4 (Wyoming)	21	0	16.2%
(Apelawa× Apelawa)	CBWYO-5 (Wyoming)	18	0	63.6%
(Apelawa× Marangani)	CBWYO-6 (Wyoming)	6	1	31.6%
(Apelawa× Apelawa)	CBWYO-8 (Wyoming)	1	7	20.6%
(Apelawa× Marangani)	CBWYO-9 (Wyoming)	20	0	25.9%
(Apelawa× Marangani)	CBWYO-10 (Wyoming)	20	0	26.9%
(Apelawa× Marangani)	SLV-1 (Colorado)	20	0	21.3%
(Apelawa× Marangani)	SLV-2 (Colorado)	0	6	–

*Mean of five plants sampled.

Table 6. Segregation among F_2 progeny from three male fertile F_1 *C. quinoa* × *berlandieri* hybrids

F_1 parent	Progeny		
	Male fertile*	Intermediate**	Male sterile
(Apelawa/Marangani × CBWYO-1)	7	16	34
(Apelawa/Apelawa × CBWYO-8)	7	28	17
(Apelawa/Marangani × SLV-1)	9	18	33

* Anthers present in >90% of flowers, pollen viability <85%.
** Anthers present in 10–60% of flowers, pollen viability <65%.

tween the *C. quinoa* and *C. berlandieri* genomes which are not related to the presence of a male sterile cytoplasm.

The morphology and partial restoration of fertility observed in known *C. quinoa* × *berlandieri* hybrids suggests that AP12, AP14, AP18 and AP30, the four original 'Apelawa' accessions which produced mixtures of male sterile and male fertile F_1 offspring, were themselves male sterile plants which had received pollen from more than one source. Pollen donors may have included not only adjacent male fertile quinoa plants, but also *C. berlandieri*, which was present and plentiful in the original 'Apelawa' field population. In addition to segregation for male sterility and fertility, considerable morphological variation was observed among the first generation of offspring derived from these four accessions. Some progeny resembled the maternal 'Apelawa' parent, with single main stems and compact amarantiform inflorescences, while others were distinctly weedy in appearance with loose glomerular inflorescences and branching stems. Plants in this generation which possessed anthers were invariably of this weedy morphological type, and their resemblance to the known *C. quinoa* × *berlandieri* hybrids grown later in the course of this work was striking. Isozyme analysis has indicated that gene flow does occur between *C. quinoa* and *C. berlandieri* in the field (Wilson, 1991), and our observations here suggest that such gene movement may be facilitated where male sterile quinoa plants are present. The potential introgression of undesirable weed genes from *C. berlandieri* into commercial quinoa populations has not previously been considered with respect to crop management, but we would recommend more vigorous control of *C. berlandieri* by growers, especially those who save their own seed for future plantings.

The cytoplasmic male sterility we have identified appears to be stable in combination with quinoa nuclear genomes other than 'Apelawa', as indicated by the crosses using various South American cultivars as pollen parents. Plants carrying this cytoplasm are also readily identifiable at flowering due to the complete absence of anthers, and are easily pollinated while the stigmas are exserted. We suggest that these characteristics of the 'Apelawa' male sterile cytoplasm make it a potentially valuable aid in hybridizing quinoa, if a suitable restorer system can be developed. The restorer factor in *C. berlandieri* may be usable in some instances, if it can be combined with an appropriate genetic background which will maximize the extent of restoration while eliminating undesirable associated weedy characteristics. More complete restoration of male fertility in the F_1 generation, however, will be necessary if cytoplasmic male sterility is to be used in the commercial production of hybrid quinoa seed.

Acknowledgement

This work was supported by Colorado State University Cooperative Extension Project No. 15-7291.

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