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Production and characterization of an interspecific hybrid between leek and garlic

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Abstract An interspecific hybrid between leek (*Allium ampeloprasum* L.) and garlic (*Allium sativum* L.) was produced by hybridization using a fertile garlic clone as a pollen donor and an ovary culture. The hybridity was confirmed by chromosome observation ($2n = 3x = 24$) and randomly amplified polymorphic DNA (RAPD) analysis. The interspecific hybrid showed a vigorous growth habit, and its foliage was larger than that of the parental species. The bulb of the interspecific hybrid was heavier than that of the parents, containing an intermediate number of cloves. The hybrid could be propagated vegetatively by planting cloves. The odor compounds of garlic, which leek did not have, were detected in the volatiles of the hybrid with a certain concentration. The results of the study suggest the possibility of direct use of an interspecific hybrid between *A. ampeloprasum* and *A. sativum* as a new crop.

Keywords Leek · Garlic · Interspecific hybridization

Introduction

The genus *Allium* includes many vegetable crops which have a long history of cultivation. Attempts for their genetic improvement involve not only intraspecific but also interspecific hybridization. Interspecific hybridization is a useful tool to introduce useful genes or to produce new crops. Recent attempts of interspecific hybridization in edible *Allium*, especially between onion and other species, have generated new kinds of breeding materials successfully (Keller et al. 1996; Peffley and Hou 2000).

Leek (*Allium ampeloprasum* L.) is a seed-propagated plant whose leaves are used as a vegetable. Although its foliage is harvested commonly in autumn or winter, leek has the potential to form a bulb in the following summer. Garlic (*Allium sativum* L.) is a vegetatively propagated plant whose bulb is used as a spice. Although most cultivated garlic clones are sterile, Etoh (1986) found fertile clones in Central Asia. Leek and garlic are closely related and classified into the same subgenus *Allium*. Leek has disease resistance which garlic does not have (Porter and Jones 1932), while garlic possesses specific odor compounds. Interspecific hybridization between the two species is expected to lead to the improvement of leek and garlic, or to generate a new *Allium* crop. So far, no hybrids between the two species have been reported except by a preliminary study of Sugimoto et al. (1991).

In the present study, an interspecific hybrid between leek and garlic was produced and characterized. Foliage and bulb characteristics and odor compounds of the hybrid are described in comparison with the parental species. The possibility of direct use of the hybrid between the two species is discussed.

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Materials and methods

Plant materials

Leek cv Wonder (Kyowa Seed Co.) was used as a female parent. The pollen parent, a fertile garlic clone RAR930064, was provided by the Laboratory of *Allium* Vegetables Breeding, the National Research Institute of Vegetables, Ornamental Plants and Tea. The garlic clone was collected from Kirghizstan, and listed in the Genebank, National Institute of Agrobiological Sciences, with the JP number of 134667.

Pollination and ovary culture

In the summer of 1998, leek flowers were emasculated and pollinated with garlic pollen. The crossing was performed indoors using cut flowers of leek. Four to six days after pollination, ovaries were separated and surface-sterilized in 70% ethanol and 1% sodium hypochlorite solution. After being rinsed in sterile distilled water, they were placed on LS agar medium (Linsmaier and Skoog 1965) supplemented with 5% sucrose. The ovary culture was performed at 20 °C with a 14-h photoperiod of fluorescent light (about 2,500 lux). After 52 days of culture, plantlets that developed from an ovary were transferred to a new medium containing 3% sucrose and cultured with a 12-h photoperiod.

In the spring of 1999, the cultured plants were transferred to pots, and in the autumn they were transplanted to soil in a greenhouse. The plants passed the winter without heating, and their bulbs were harvested in the summer of 2000.

Chromosome observation

Root tips of the potted plants were collected and treated with 0.5 mM of 8-oxyquinoline for 21 h at 0 °C. After the root tips were fixed in ethanol/acetic acid (3:1), chromosomes were stained with aceto-orcein and observed by a squash method.

RAPD analysis

Total DNA was extracted from the leaf segment using a DNeasy Plant Mini Kit (Qiagen Inc.). RAPD patterns were compared using primers OPB-01, OPD-01 and OPH-01 (Operon Technologies Inc.). Amplifications were carried out in 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 400–800 nM primer, 1 unit of *Taq* polymerase (Takara Shuzo Co.) and 30–100 ng of template DNA per 20-μl reaction. Initial denaturation was performed for 3 min at 94 °C, followed by 45 cycles of 1 min at 94 °C, 1 min at 38 °C, 2 min at 72 °C, and a final 5-min extension at 72 °C using Takara PCR Thermal Cycler MP. The amplified products were electrophoresised in 2% agarose gel and stained with ethidium bromide.

Field experiments

On October 8, 2000, cloves of the hybrid and the parental species were planted in a field at the Aomori Field Crops and Horticultural Experiment Station. The ridge was mulched with a colorless polyethylene sheet, and the cloves were planted in rows, 15-cm apart and 25-cm between each row. The fertilizer application was 2.5 kg of N, 3.0 kg of P_2O_5, and 3.9 kg of K_2O per are=100 m^2.

Bolting flower stalks of the hybrid plants and their parents were cut off in order to nourish their bulbs. A few plants of the hybrid were allowed to flower and their pollen fertility was examined by aceto-carmine stainability.

On July 15, 2001, the plants were harvested, and the length of the longest leaf was measured from the bottom of the bulb. After cutting off their tops and roots, bulbs were air-dried using a heated drier at about 35 °C. The bulbs were weighed when their water content was about 70%.

Analysis of odor compounds

Cloves of the bulb were chopped into 3–5-mm cubic pieces after their protective leaf was removed. Ten grams of the chopped cloves were soaked in 100 ml of 20% ethanol overnight at 5 °C. Volatile compounds were absorbed into a Tenax TA column, and eluted with ether by the method of Shimoda et al. (1987). The ether solution was dehydrated with Na_2SO_4 overnight and concentrated by evaporation. Diethyl disulfide was added to the solution as an internal standard.

The odor concentration was analyzed by gas chromatography using Shimadzu GC-14A equipped with an HP-INNOWax column (0.25 mm i.d. × 60 m). The operating conditions were as follows: oven temperature, 40 °C for 5 min, 3 °C /min rising to 200 °C; carrier gas, helium; flow rate, 0.8 ml/min; injection temperature, 210 °C; detector, FID (210 °C) and FPD (230 °C). Concentration of the major sulfur compounds was calculated from the peak area ratio to the internal standard in the retention time of 10–50 min. The peak identification was confirmed by mass spectrometry coupled to gas chromatography using Hewlett Packard 5890 and Hewlett Packard 5972 with an ionization voltage of 70 eV.

Results

Production of hybrid plants

Among the 87 cultured ovaries, only one generated a plantlet after about 7 weeks of culture (Fig. 1A). As the plantlet had two apical meristems at both terminals of a fistulous leaf, it was divided into two and subcultured. One more adventitious bud appeared from a root tip of one cultured plant after subculturing. Finally, three plants which were thought to originate from a single ovule were potted. These plants were transplanted into a greenhouse and each formed a bulb in the following summer.

Confirmation of hybridity

Root-tip cells of all the three plants showed 24 chromosomes in their mitosis (Fig. 1B). This is in agreement with the sum of the haploid chromosome numbers in leek (n = 16) and garlic (n = 8).

RAPD patterns of leek and garlic could be clearly distinguished with all the primers used. RAPD patterns of the cultured plants were investigated using vegetatively propagated progenies, and they had both the specific bands of leek and garlic (Fig. 1C).

Growth habit and morphology

Foliage of the interspecific hybrid had keeled leaves, which showed an intermediate plant shape between leek and garlic plants (Fig. 1E). The plant size of the hybrid resembled leek, but was more vigorous. The average weight of the planted seed cloves was 7.7 g for leek, 5.1 g for garlic and 4.8 g for the interspecific hybrid. Despite the small seed cloves, the longest leaf of the hybrid was significantly longer than that of the parents (Table 1).

Bolting of the hybrid and leek was observed in late May, whereas garlic bolted in the middle of June. Secondary flower stalks also bolted from the leaf axil of the hybrid. The inflorescence of the hybrid showed an umbel form similar to leek which did not contain bulbils (Fig. 1D). The tepal color of the hybrid was pink, which is a character of the fertile garlic. Almost no fertile pollen grains were observed in anthers of the hybrid.

Only half of the leek plants formed a bulb, while all the plants formed a bulb in the garlic and the hybrid.

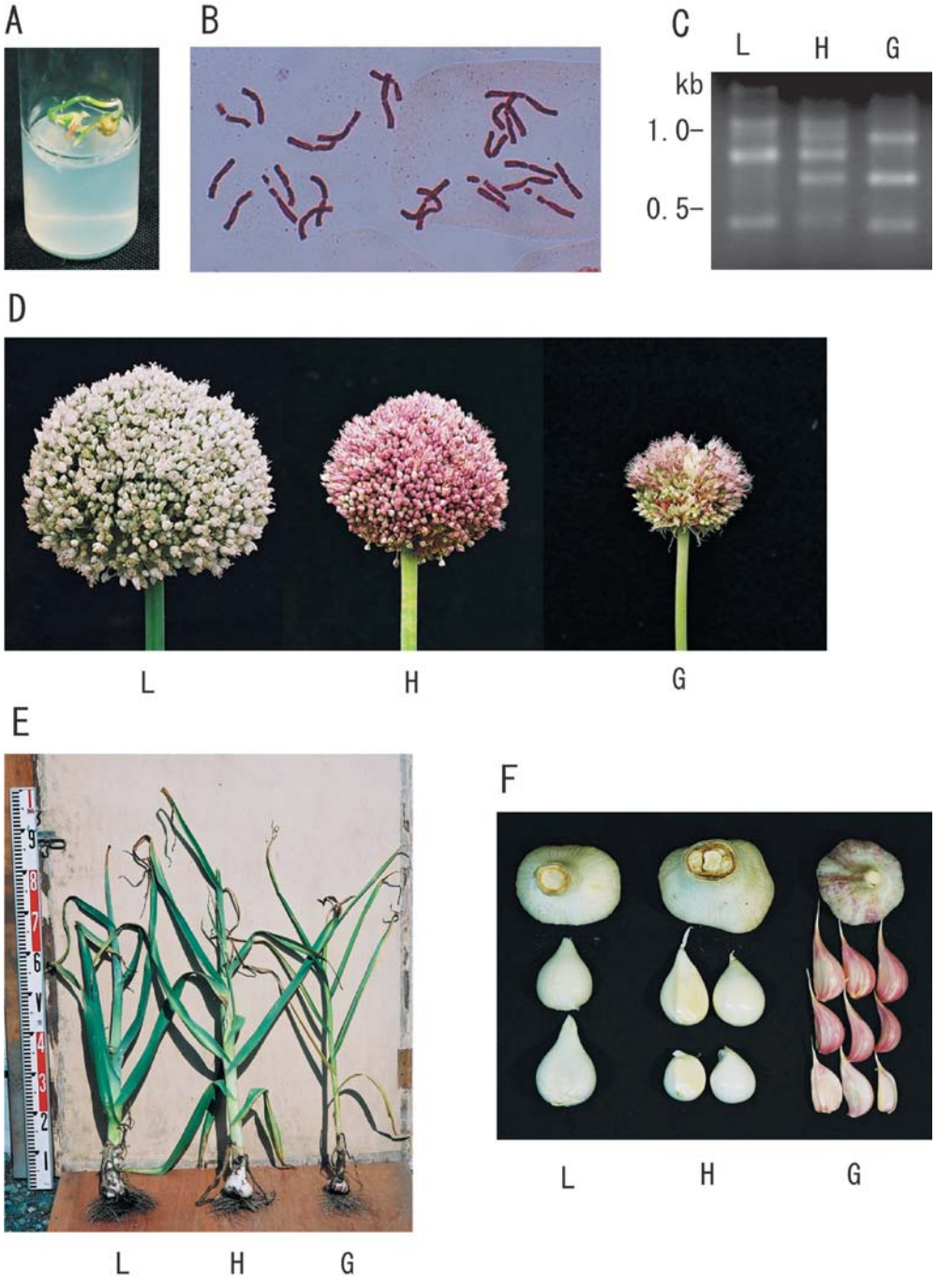

Fig. 1A–F **A** Plantlet generated from a cultured ovary. **B** Chromosomes of the interspecific hybrid plant (2n = 3*x* = 24). **C** RAPD pattern with primer OPB-01 of leek (*L*), garlic (*G*) and the interspecific hybrid (*H*). **D** Inflorescence of leek (*L*), garlic (*G*) and the interspecific hybrid (*H*). **E** Plant of leek (*L*), garlic (*G*) and the interspecific hybrid (*H*). **F** Bulb and cloves of leek (*L*), garlic (*G*) and the interspecific hybrid (*H*)

Table 1 Foliage and bulb characteristics of leek, garlic and the interspecific hybrid. ± indicates standard deviation

	No. of plants examined	Length of the longest leaf (cm)	No. of plants which formed a bulb	Bulb weight (g)	No. of cloves per bulb
Leek	9	99.7 ± 9.8	5	65.8 ± 19.4	1.8 ± 0.4
Garlic	16	97.6 ± 9.7	16	45.8 ± 12.6	9.3 ± 0.9
Hybrid	13	113.6 ± 9.0	13	70.1 ± 13.1	4.0 ± 0.6

Table 2 Concentration (ppm/fresh weight) of volatile sulfur compounds in chopped cloves of leek, garlic and the interspecific hybrid. –: not detected

	Allyl methyl disulfide	Allyl(E)-1-propenyl disulfide	Diallyl disulfide	2-Vinyl-4H-1,3-dithiin	3-Vinyl-4H-1,2-dithiin
Leek	–	–	–	–	–
Garlic	18.4	26.3	101.9	172.2	47.9
Hybrid	10.2	19.8	25.8	44.3	10.2

Bulbs of the hybrid were larger and heavier than those of the parents (Table 1, Fig. 1F). The number of cloves per bulb was 1 or 2 in the leek plants, while ranging from 8 to 11 in the garlic plants. A bulb of the hybrid contained 3 to 5 cloves, whose number was an intermediate value between the parents. Although the fertile garlic parent has red pigment in its protective leaf of the bulb, such a pigment was not expressed in the hybrid.

Content of odor compounds

Leek showed only a few low peaks, whose highest concentration was 1.4 ppm. The major compounds detected in garlic were three allylic disulfides and two vinyldithiins, which were not found in leek (Table 2). The interspecific hybrid also had the five compounds detected in garlic as major peaks. Each concentration of diallyl disulfide and two vinyldithiins was about a quarter of the amount in comparison to garlic, whereas two asymmetrical disulfides contained more than half the amount of garlic.

Discussion

A considerable number of interspecific hybrids have been produced thus far in edible *Allium*, most of which have been generated from the combination of onion with other species (Gonzalez and Ford-Lloyd 1987, Keller et al. 1996). Peterka et al. (1997) obtained the hybrid between onion and leek, while Ohsumi et al. (1993a) succeeded in the hybridization between onion and fertile garlic.

Concerning the hybridization between leek and garlic, a preliminary attempt has been reported by Sugimoto et al. (1991) for the purpose of introducing high fertility into garlic. Although they used seven cultivars of leek and seven lines of fertile garlic, and obtained triploid and aneuploid plants by embryo culture, characteristics of the hybrids were not reported. In the present study, the hybridity between the two species was confirmed at the molecular, cytological and morphological levels. The interspecific hybrid grew vigorously, formed a bulb, and was propagated vegetatively. The foliage and bulb of the hybrid showed intermediate characteristics between the parental species.

In this study, we could obtain the interspecific hybrid through ovary culture. In the recent work of interspecific hybridization in *Allium*, most employed embryo rescue techniques except for Buiteveld et al. (1998) who succeeded in somatic hybridization between leek and onion. Nomura and Makara (1993) compared some embryo rescue techniques in the hybridization between Rakkyo (*Allium chinense* G. Don) and the bunching onion (*Allium fistulosum* L.), and reported the effectiveness of ovary culture. Keller et al. (1996) also exclusively adopted ovary culture and obtained many hybrids between onion and other distant species. These results suggest the extensive utility of ovary culture for producing interspecific hybrids in the genus *Allium.*

Diallyl disulfide and vinyldithiins are derived from allicin (diallyl thiosulfinate), which is the unstable source of garlic odor (Kim et al. 1995). Leek has been reported not to have allicin and other allylic thiosulfinates (Block et al. 1992). Our results indicated the presence of allicin derivatives in the volatiles of the interspecific hybrid. Actually, the chopped cloves of the hybrid had a mild odor similar to garlic. Ohsumi et al. (1993b) also reported the presence of diallyl disulfide in an interspecific hybrid between onion and garlic. These results indicate that the synthetic pathway of alliin (S-allylcysteine S-oxide), which is the precursor of allicin, and the activity of alliinase, which catalyzes the conversion to odor, are retained through interspecific hybridization.

In contrast to the trial for the introduction of desirable traits such as disease resistance (Peffley and Hou 2000) another purpose of interspecific hybridization is the direct use of a hybrid. In Japan, new *Allium* vegetables have been released from the interspecific hybridization between bunching onion and Chinese chive (Amagai et al. 1995), and between Rakkyo and other *Allium* species (Nomura and Makara 1993, 1996). As for the interspecific hybrid between leek and garlic, the vigorous growth habit and the ability of vegetative propagation suggested the possibility of direct use.

The *A. ampeloprasum* complex consists of various genetic resources including leek, kurrat and great-headed garlic (Kik et al. 1997). Fertile garlic clones also possess considerable genetic variation (Hong and Etoh 1996). The interspecific hybrid between *A. ampeloprasum* and *A. sativum* could be expected to become a new *Allium* crop propagated vegetatively. Extensive crossing involving various materials should be considered, and a new crossing is currently being carried out.

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