Genetic Mapping of a Dwarfing Gene Found in *Solanum phureja* Clone 1.22

Takafumi Kimura and Kazuyoshi Hosaka*

Experimental Farm, Kobe University, 1348 Uzurano, Kasai, Hyogo 675-2103, Japan.
*Corresponding author: Tel: 81-790-49-3121; Fax: 81-790-49-0343,
E-mail: hosaka@kobe-u.ac.jp

ABSTRACT

Dwarf plants were obtained in an F_2 population of a cross between *Solanum chacoense* (clone chc 525-3) and *S. phureja* (clone 1.22). Segregation analyses in F_2 and backcross populations suggested that the dwarfism was controlled by a single recessive gene transmitted from *S. phureja* clone 1.22. The dwarf plants responded to treatment with gibberellic acid, which recovered normal growth. We named this gene 'ga_2'. Linkage analysis of the gene ga_2 with RFLP and RAPD markers indicated that the gene ga_2 was located on the most or near distal end of chromosome 7.

RESUMEN

Del cruce entre *Solanum chacoense* (clon 525-3) y *S. phureja* (clon 1.22) en una población F_2 se obtuvieron plantas enanas. Los análisis de segregación en F_2 y las poblaciones en retrocruza sugirieron que el enanismo estuvo controlado por un solo gen recesivo trasmitido del clon 1.22 de *S. phureja*. Las plantas enanas respondieron al tratamiendo con ácido giberélico después de lo cual recuperaron su crecimiento normal. A este gen lo hemos denominado "ga_2". Los análisis de enlace del gen ga_2 realizados con marcadores de RFLP y RAPD indicaron que el gen ga_2 estaba localizado en la parte más extrema del cromosoma 7 o cerca de su extremo distal.

INTRODUCTION

Dwarfism has been successfully used in several cereal crops to shorten stem length and to confer lodging resistance. Many wild and cultivated potato species grow in low latitude regions in Andes (Hawkes 1990). These and their hybrid progeny with the common potato (*Solanum tuberosum* L. ssp. *tuberosum*, referred to those presently grown worldwide) tend to grow vigorously without initiating tuber formation due to long day-length in summer season in medium latitude regions. Dwarfism may contribute to some extent to improving vine shape of potato.

Dwarf plants in potato may be commonly appearing in practical breeding programs, but virtually all such plants are eliminated in a very early stage of selection. Hermsen et al. (1978) obtained extreme dwarf plants with single stems carrying many small, generally simple leaves on relatively long petioles, in a selfing population of a haploid clone of *S. tuberosum* ssp. *tuberosum* cv. Gineke. They assumed the dwarf trait to be controlled by a single recessive gene (Hermsen et al. 1978). In progeny of a cross between *S. tuberosum* ssp. *andigena* and ssp. *tuberosum*, Bamberg and Hanneman (1991) found dwarf plants having very short internodes, small and dark green leaves and a compact ball-shaped appearance which could be completely restored to normal appearance by exogenous GA_3. By test crosses, a single recessive gene 'ga_1' was proposed to explain this dwarfism (Bamberg and Hanneman 1991). The block of gibberellin biosynthesis between GA_{12} and GA_{53} in this dwarf plant has been found (van den Berg et al. 1995). Significant yield differences were also reported for tetraploid ga_1 heterozygotes that show no obvious vine differences (Bamberg and Hanneman 1993). Dwarf plants having the same morphological characteristics with Bamberg and Hanneman's (1991) were reported in a haploid population of *S. tuberosum* ssp. *tuberosum* cv. Pito (Valkonen et al. 1999). They measured endogenous gibberellin and found very low amounts of all analyzed GAs in the dwarfs, indicating an early part of the gibberellin biosynthesis pathway is blocked. They gave a gene symbol '*pito*' to a recessive dwarfing gene without a genetic analysis.

We obtained extreme dwarf plants in an F_2 population of a cross between a wild diploid species, *S. chacoense* (clone chc

Accepted for publication December 19, 2001.
ADDITIONAL KEY WORDS: Gibberellin sensitive, recessive gene, potato, RFLP, RAPD.

525-3), and a cultivated diploid species, *S. phureja* (clone 1.22). In this paper, we report the results of genetic analysis of the dwarfism and the mapping of a dwarf gene.

MATERIALS AND METHODS

Solanum chacoense clone chc 525-3 is a highly inbred line (S_7) obtained through continued selfings utilizing S locus inhibitor gene (*Sli*) (Hosaka and Hanneman 1998a). *Solanum phureja* clone 1.22 is known as a superior diploid pollinator from PI 225682 commonly used in haploid extraction in North America (Kotch and Peloquin 1987). One of F_1 hybrids between chc 525-3 and 1.22 (clone F_1-1) was selfed with the help of *Sli* gene (2KH74 family), or backcrossed to both parents (2KH72 and 2KH73 families) (Figure 1). F_1-1 was crossed with a cultivated diploid clone 93H100-1 (94H89 family). A randomly chosen individual from 94H89 family was selfed (1H76 family). A dwarf plant 90H29-173 obtained by selfing F_1-1 was backcrossed to F_1-1 (2KH75 family).

Seeds were soaked in 2000 ppm GA_3 for 48 h and sown in cell pots individually. Young seedlings were transplanted to 4 inch clay pots 35 days after seed sowing. A further 35 days later, dwarf plants were distinguished. After determination of phenotypes, some dwarf plants of 2KH74 family were sprayed with 50 ppm GA_3 three times a week for 3 wk to test whether they recovered to normal growth or not.

Plants of 2KH74 family were used for mapping a dwarfing gene. DNA isolation and detection procedures for RAPD and RFLP markers have been described previously (Hosaka and Hanneman 1998b). RFLP probes prefixed with 'TG', 'CT' or 'CD' were single-copy tomato probes obtained from S. D. Tanksley, Cornell University, NY, USA. These have been localized on tomato or potato genetic maps (Tanksley et al. 1992). The probes prefixed with 'P' were single-copy DNA probes selected from a random genomic DNA library of *S. phureja* 1.22 (Hosaka and Spooner 1992), which have been localized on potato genome (Hosaka and Hanneman 1998b; Hosaka 1999). Linkage analysis was carried out by the program 'MAPMAKER' (Lander et al. 1987) with Kosambi mapping function.

RESULTS AND DISCUSSION

Parental clones chc 525-3 and 1.22 had normal appearance. F_1 hybrids were also all normal. One of the F_1 hybrids, F_1-1, was selfed, and extreme dwarf plants were found in a ratio of 120 normal : 37 dwarf in the selfed progeny. Dwarf plants had very short internodes, small dark green leaves with edges of leaflet often curled to upside, and compact ball-shape appearance (Figure 2), which looked like the dwarfs of Bamberg and Hanneman (1991) and Valkonen et al. (1999). F_1-1 was backcrossed to chc 525-3 and to 1.22, and the latter yielded dwarf plants in a ratio of 199 normal : 63 dwarf (Table 1), indicating that the dwarfism was inherited from 1.22. These and other segregation ratios (Table 1) suggest that the dwarfism is controlled by a single recessive gene.

By spraying GA_3, complete recovering to normal growth was observed in many of the dwarfs. Some did not recover probably because very poor root system could not sustain rapid aboveground growth.

Morphological features, sensitiveness to exogenous gibberellin, and a single recessive genic action in the present dwarfs suggest a high similarity of the present dwarfing gene to previously reported ga_1 or *pito*. Since allelism tests of these genes are not completed, we herein designate our gene to be 'ga_2'. The pos-

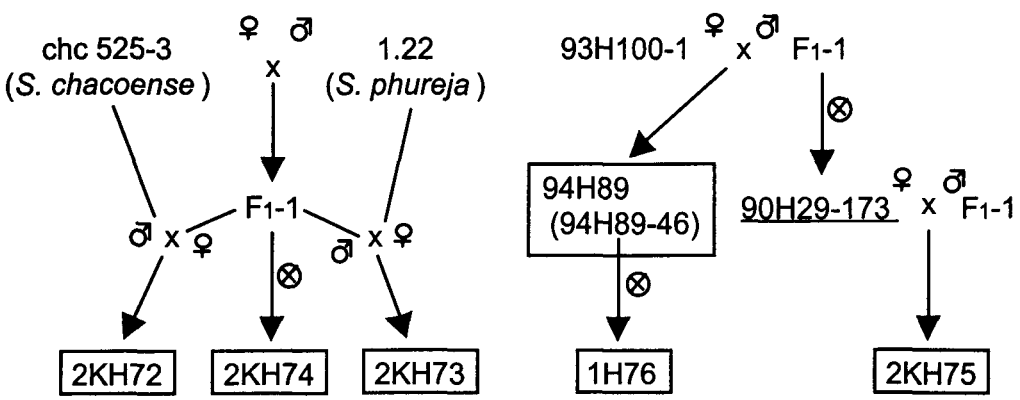

FIGURE 1.
Materials used in this study. Populations are represented in boxes. An underlined individual is a dwarf plant. ⊗ indicates selfing.

tulated genotypes for 1.22 and F_1-1 are both $Ga_2 ga_2$. Dwarfing genes ga_1 and *pito* were found in *S. tuberosum* ssp. *andigena* and ssp. *tuberosum*, respectively. The present dwarfing gene ga_2 was found in *S. phureja*. However, all these species are closely related to cultivated potatoes (Hawkes 1990). Thus, the same type of dwarfing genes may be distributed widely in the cultivated potato gene pool. Bamberg (1999) reported that a GA-deficiency-type dwarfing gene, or ga_1, was not particularly rare in *S. tuberosum* ssp. *andigena* collections of the US Potato Genebank.

FIGURE 2. General appearance of normal (left) and dwarf (right) plants.

Dwarf plants rarely flowered and have not been pollinated because of the low availability of flowers except for 90H29-173, which was successfully crossed with F_1-1 resulting in 2KH75 family. The proportion of dwarfs in 2KH75 family was lower than the expected 1:1 ratio at a 5% significance level (Table 1). Also, in the other families where the 3:1 ratio was expected, lower frequencies of dwarfs were obtained. A much higher frequency (36.6%) of the number of seedlings in 2KH75 family perished before the phenotype determination, compared with 9.8-13.8% in the other families. Thus, it can be suggested that the ga_2 ga_2 individuals are less likely to survive than are Ga_2Ga_2 or Ga_2ga_2.

Using 96 normal and 14 dwarf plants of 2KH74 family, linkage analysis was performed with RFLP and RAPD markers and a dwarfing gene ga_2. With a LOD score value of 5.0, 15 RFLP markers and three RAPD markers formed a linkage group that contained the dwarfing gene ga_2 located on one of the most distal ends (Figure 3). For example, three recombinants (two dwarf and one normal) and eight recombinants (three dwarf and five normal) in a total of 110 plants were detected between TG13A and ga_2 and between CT52 and ga_2, respectively. Locations of two RFLP loci P703 and P73 and two 1.22 specific RAPD markers 144-760 and 139-1200 are first reported in this study. All other RFLP and RAPD markers have been localized on chromosome 7 previously (Tanksley et al. 1992; Hosaka and Hanneman 1998b; Hosaka 1999). According to Tanksley et al. (1992), TG13A locus is located on the end of a potato map and both TG13A and CT52 are located at the same position at the end of a tomato map. P908 is located on near distal end of chromosome 7 (Hosaka and Hanneman 1998b). Thus, it is suggested that the dwarfing gene ga_2 is located on the most or near distal end of chromosome 7. Tight linkage of ga_2 with TG13A and CT52 was also confirmed by linkage analysis using 1H76 family (data not shown).

TABLE 1—*Phenotypic segregation for normal vs dwarf appearance.*

Family	Pedigree	Postulated genotypes	Observed		Expected ratio
			Normal	Dwarf	
2KH72	F_1-1 x chc 525-3	Ga_2ga_2 x Ga_2Ga_2	46	0	1:0 ns
2KH73	phu 1.22 x F_1-1	Ga_2ga_2 x Ga_2ga_2	199	63	3:1 ns
2KH74	F_1-1 self	Ga_2ga_2 self	120	37	3:1 ns
2KH75	90H29-173 x F_1-1	ga_2ga_2 x Ga_2ga_2	52	31	1:1*
94H89	93H100-1 x F_1-1	Ga_2Ga_2 x Ga_2ga_2	116	0	1:0 ns
1H76	94H89-46 self	Ga_2ga_2 self	136	36	3:1 ns

Observed ratios were tested against expected ratios by Chi square test. ns: not significant, *: significant at a 5% level

Bamberg and Hanneman (1993) found that the ga_1 gene had a dose effect on tuber yield; yields in tetraploids followed the pattern of simplex ($Ga_1ga_1ga_1ga_1$) > duplex ($Ga_1Ga_1ga_1ga_1$) > triplex ($Ga_1Ga_1Ga_1ga_1$). If this is generally true, the assessment of dwarfing locus would become important in potato breeding. In their study, test crossing and progeny evaluation determined the genotypes. Alternatively, RFLP markers can rapidly determine genotypes: for example, if two RFLP bands A and B are tightly linked to Ga_1 and ga_1, respectively, ratios of band intensities would be A<B for simplex, A=B for duplex and A>B for triplex. Thus, linked markers TG13A and CT52 in this study

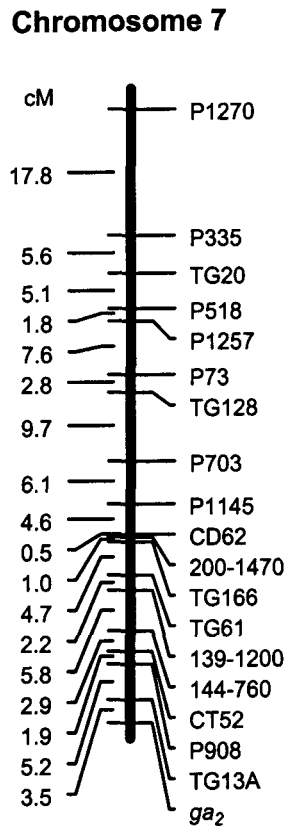

FIGURE 3.
Linkage relationships between a dwarfing gene (ga_2) and DNA markers located on chromosome 7. Markers started with figures are 1.22 specific RAPD markers shown by a primer number hyphened with the size of the marker band in base pair.

would be useful to conduct the same type of experiments for evaluating the dosage effect of ga_2, and may be widely effective in assessing the usefulness of this locus in a breeding program.

ACKNOWLEDGMENTS

We thank J. B. Bamberg, USDA, and K. Kawano, Kobe University, for reading of the manuscript and useful comments. We also thank M. Miyake, T. Yamashita, S. Kakehi, M. Fujimatsu, and S, Kitanishi for plant maintenance. This work was supported in part by Nagasaki Pref. Agricultural & Forestry Experiment Station, Nagasaki, Japan.

LITERATURE CITED

Bamberg, J.B. 1999. Screening for gibberellin deficiency mutants in *Solanum tuberosum* ssp. *andigena*. Am J Potato Res 76:321-322.

Bamberg, J.B., and R.E. Hanneman, Jr. 1991. Characterization of a new gibberellin related dwarfing locus in potato (*Solanum tuberosum* L.). Am Potato J 68:45-52.

Bamberg, J.B., and R.E. Hanneman, Jr. 1993. Transmission and yield effects of a gibberellin mutant allele in potato. Potato Res 36: 365-372.

Hawkes, J.G. 1990. The Potato–Evolution, Biodiversity and Genetic Resources. Belhaven Press, London.

Hermsen, J.G.Th., L.M. Taylor, E.W.M. van Breukelen, and A. Lipski. 1978. Inheritance of genetic markers from two potato dihapoids and their respective parent cultivars. Euphytica 27:681-688.

Hosaka, K. 1999. A genetic map of *Solanum phureja* clone 1.22 constructed using RFLP and RAPD markers. Am J Potato Res 75: 97-102.

Hosaka, K., and R.E. Hanneman, Jr. 1998a. Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. I1 Detection of an S locus inhibitor (Sli) gene. Euphytica 99:191-197.

Hosaka, K., and R.E. Hanneman, Jr. 1998b. Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. 2. Localization of an S locus inhibitor (Sli) gene on the potato genome using DNA markers. Euphytica 103:265-271.

Hosaka, K., and D.M. Spooner. 1992. RFLP analysis of the wild potato species, *Solanum acaule* Bitter (*Solanum* sect. *Petota*). Theor Appl Genet 84:851-858.

Kotch, G.P., and S.J. Peloquin. 1987. A new sources of haploid germplasm for genetic and breeding research. Am Potato J 64:137-141.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Tanksley, S.D., M.W. Ganal, J.P. Prince, M.C. de Vicente, M.W. Bonierbale, P. Broun, T.M. Fulton, J.J.Giovannoni, S. Grandillo, G.B. Martin, R. Messeguer, J.C. Miller, L. Miller, A.H. Paterson, O. Pineda, M.S. Röder, R.A. Wing, W. Wu, and N.D. Young. 1992. High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160.

Valkonen, J.P.T., T. Moritz, K.N. Watanabe, and V.-M. Rokka. 1999. Dwarf (di)haploid *pito* mutants obtained from a tetraploid potato cultivar (*Solanum tuberosum* subsp. *tuberosum*) via anther culture are defective in gibberellin biosynthesis. Plant Sci 149:51-57.

van den Berg, J.H., I. Simko, P.J. Davies, E.E. Ewing, and A. Halinska. 1995. Morphology and [^{14}C]gibberellin A_{12} metabolism in wild-type and dwarf *Solanum tuberosum* ssp. *andigena* grown under long and short photoperiods. J Plant Physiol 146:467-473.