Theor Appl Genet (1991) 81:376–380



# Identification and genetic analysis of semidwarfism-related proteins in rice (*Oryza sativa* L.)

**A. Nakamura [1], H. Hirano [2,*] and F. Kikuchi [1]**

[1] Institute of Plant Breeding, University of Tsukuba, Ibaraki 305, Japan
[2] Department of Molecular Biology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki 305, Japan

Received July 7, 1990; Accepted August 22, 1990
Communicated by G. S. Khush

**Summary.** By transferring a semidwarf gene (*sd-1*) from Taichung Native 1 into a tall Japanese cultivar, Norin 29, through seven backcrosses, a semidwarf near-isogenic line SC-TN1 was obtained. The proteins of the embryo in Norin 29 and SC-TN1 were separated by two-dimensional electrophoresis. Most of the proteins showed the same electrophoretic pattern. However, it was found that there was a difference in the appearance of two basic glycoproteins designated as SRP-1 and SRP-2. These proteins exhibited the same molecular mass, but different isoelectric points. Hybridization results indicated that a single locus controls SRP-1 and SRP-2 with codominant alleles. The gene symbol *Srp* was given to this locus, with alleles *Srp-1* and *Srp-2* responsible for SRP-1 and SRP-2, respectively. *Srp-2* was found in all of the semidwarf cultivars and lines having *sd-1*, except a tall cultivar Tsai-yuan-chung. This finding suggests that *Srp-2* may be closely linked with *sd-1*. The amounts of these proteins markedly increased after water absorption of the seed, suggesting that these proteins may be related to the early development of the plant.

**Key words:** Rice – *Oryza sativa* – Embryo protein – Genetic analysis – Semidwarfism

## Introduction

Breeding of semidwarf cultivars is one of the most important objectives in rice improvement programs all over the world. A semidwarf Taiwanese cultivar, Taichung Native 1 (TN1), derived from a cross between a semidwarf Chinese cultivar, Dee-geo-woo-gen (DGWG), and a tall Taiwanese cultivar, Tsai-yuan-chung, has been frequently used as a parent. TN1 has a single recessive gene, *sd-1*, for the semidwarf phenotype inherited from DGWG (Aquino and Jennings 1966; Athwal 1971; Hargrove 1979).

In 1985, Kikuchi et al. produced a semidwarf near-isogenic line by four backcrosses, using a tall Japanese cultivar Norin 29 as a recurrent parent and TN1 as a donor parent. A crossing test confirmed that *sd-1* from TN1 had been transferred to the near-isogenic line (Kikuchi et al. 1985). The phenotypic characteristics of the line, such as heading date, panicle length, grain length, and seed fertility, were almost identical with those of Norin 29, except that the near-isogenic line was short-statured (Kikuchi et al. 1985).

We speculated that there may be a difference between Norin 29 and its near-isogenic line in the composition of the proteins, which are primary gene products, and that the difference may contribute to semidwarfism. If we could identify the proteins related to semidwarfism, DNAs encoding the semidwarf phenotype could be easily cloned by using advanced gene manipulation techniques.

In the present study, we separated the total proteins from mature and germinating embryos in Norin 29 and SC-TN1 by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). SC-TN1 was obtained by making three additional backcrosses of Norin 29 to the semidwarf near isogenic line produced by Kikuchi et al. (1985). We identified the proteins that showed differences in the electrophoretic patterns between Norin 29 and SC-TN1. These proteins were characterized biochemically and the genes controlling them were identified by a crossing test.

* To whom correspondence should be addressed

## Materials and methods

*Plant material*

Rice (*Oryza sativa* L.) cultivar Norin 29 and its semidwarf near-isogenic line, SC-TN1, and cultivars DGWG, Tsai-yuan-chung, and TN1 were used in this study.

*Two-dimensional gel electrophoresis*

A portion (1 mg) of embryos from the dry mature seeds or the seeds imersed in distilled water for 6, 12, 24, 48, or 60 h was removed, homogenized with 100 µl of lysis buffer (O'Farrell 1975) using a glass pestle in an Eppendorf tube, and centrifuged at 15,000 g for 5 min. Ten microliters of the supernatant was subjected to 2D-PAGE according to the method described by O'Farrell (1975) with slight modifications (Hirano 1982). After electrophoresis, the proteins were detected by Coomassie blue staining or silver staining (Gooderham 1984).

*Determination of relative molecular weight and isoelectric point*

The relative molecular weight and isoelectric point of the proteins separated by 2D-PAGE were determined using low-molecular-weight marker proteins and isoelectric point marker proteins (Pharmacia, Uppsala).

*Identification of glycoproteins*

The proteins separated by 2D-PAGE were electroblotted onto a polyvinylidene difluoride (PVDF) membrane filter (Millipore, Bedford) and made to react with peroxidase-coupled concanavalin A (Honen Oil, Tokyo), according to the procedure described by Kijmoto-Ochiai et al. (1985). The glycoproteins having N-linked oligosaccharide chains were detected as colored spots on PVDF.

*Measurement of the relative volume*

The seeds of Norin 29 and SC-TN1 were immersed in water for 6, 12, 24, 28, or 60 h at room temperature. The embryos removed from them were separated by 2D-PAGE. After 2D-PAGE, the relative volume of SRP-1 in Norin 29 and SRP-2 in SC-TN1 was measured by a two-dimensional spectrographic image analysis system (Quanta Scan 2D, Shimazu, Kyoto).

*Amino acid sequence analysis*

The proteins separated by 2D-PAGE were electroblotted onto PVDF and sequenced by a gas-phase protein sequencer (Applied Biosystems, 477A, Foster City), according to the method described by Hirano (1989).

## Results

Figure 1 shows the 2D-PAGE patterns of the embryo proteins which were detected by silver staining in Norin 29 and SC-TN1. A total of about 500 polypeptide spots were identified in each pattern. The electrophoretic pattern of all of the polypeptide spots except two was identical in Norin 29 and SC-TN1. Only one appreciable difference was found in the electrophoretic patterns; a spot designated as semidwarfism-related protein-1 (SRP-1) was detected intensively in Norin 29, but weakly in SC-TN1, while a spot designated as SRP-2 was identified unambiguously in SC-TN1, but weakly in Norin 29 by silver staining, as indicating in Figure 1. It was determined by 2D-PAGE that SRP-1 and SRP-2 exhibited the same molecular mass, about 32 kDa, but different isoelectric points, 7.4 and 7.7, respectively (Fig. 1).

Glycoproteins with N-linked oligosaccharide chains were detected on the protein-electroblotted PVDF membrane by using concanavalin A peroxidase reagents. Both SRP-1 and SRP-2 reacted with concanavalin A. It was suggested that these proteins are glycoproteins with N-linked oligosaccharide chains.

The seeds of Norin 29 and SC-TN1 were immersed in water for 6, 12, 24, 48, or 60 h at room temperature. After immersion, the embryos were removed from the seeds. Total proteins of the embryos were separated by 2D-PAGE. After 6 h of immersion, the amount of SRP-1 and SRP-2 markedly increased compared to the other embryo proteins, and continued to increase up to 48 h after immersion, although not drastically (Table 1). This finding suggests that the increase of the amount of these proteins may be related to the early development of the plant.

We determined whether or not SRP-2 is inherited from DGWG. Total embryo proteins were separated from TN1 and DGWG carrying the identical semidwarf gene *sd-1* by 2D-PAGE. Figure 2 shows the 2D-PAGE patterns of SRP-1 and SRP-2 in the pedigrees of SC-TN1. SRP-2 was always detected in the semidwarf rice cultivars, unlike SRP-1.

Results of the two crosses are presented in Table 2. The cross of Norin 29 (SRP-1) and SC-TN1 (SRP-2) yielded an $F_1$ progeny having both SRP-1 and SRP-2 (SRP-1/SRP-2). The $F_2$ progeny segregated into three types, having SRP-1, SRP-1/SRP-2, and SRP-2, giving an acceptable fit to a 1:2:1 ratio (Table 2).

The results of the crossing test suggest that a single locus controls SRP-1 and SRP-2 with codominant alleles. The gene symbol *Srp* was given to this locus with the alleles *Srp-1* and *Srp-2* responsible for SRP-1 and SRP-2, respectively. SRP-1/SRP-2 was considered to be controlled by the heterozygote of *Srp-1* and *Srp-2*.

**Table 1.** Change in the amounts of SRP-1 and SRP-2 proteins after water absorption of seeds

| | Relative volume | | | | |
|---|---|---|---|---|---|
| | Time (h) | | | | |
| | 0 | 6 | 12 | 24 | 48 |
| SRP-1[a] | 25.2 | 207.5 | 58.3 | 69.7 | 57.6 |
| SRP-2[b] | 31.3 | 137.0 | 194.5 | 39.8 | 79.9 |

[a] SRP-1 from Norin 29
[b] SRP-2 from SC-TN1

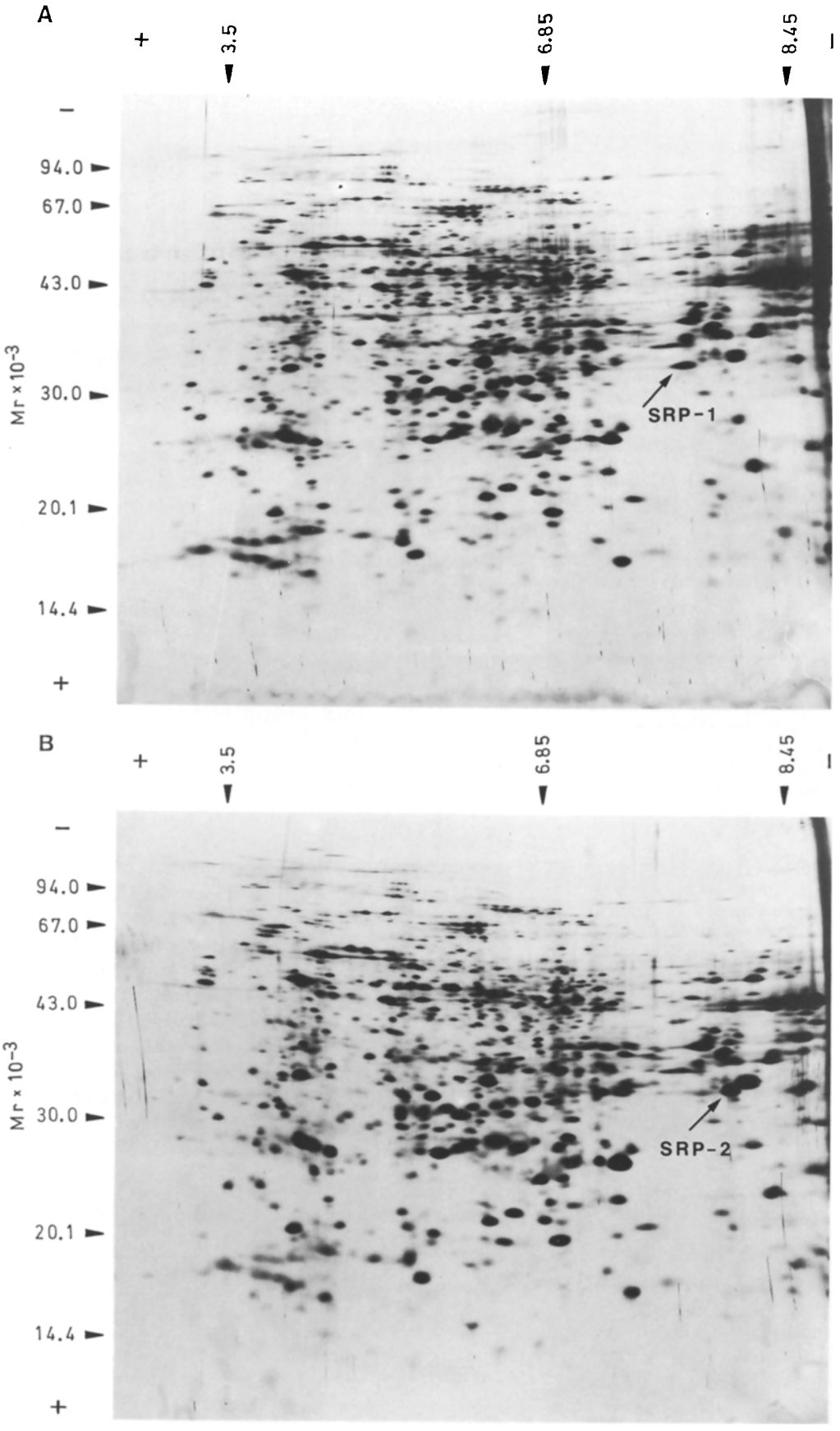


**Fig. 1A and B.** 2D-PAGE patterns of rice embryo proteins. **A** Japanese cultivar Norin 29 (*arrow* shows SRP-1); **B** semidwarf near-isogenic line SC-TN1 (*arrow* shows SRP-2). *Right* to *left*, isoelectric focusing for first dimension; up to down, SDS-PAGE for second dimension. Detected by silver staining

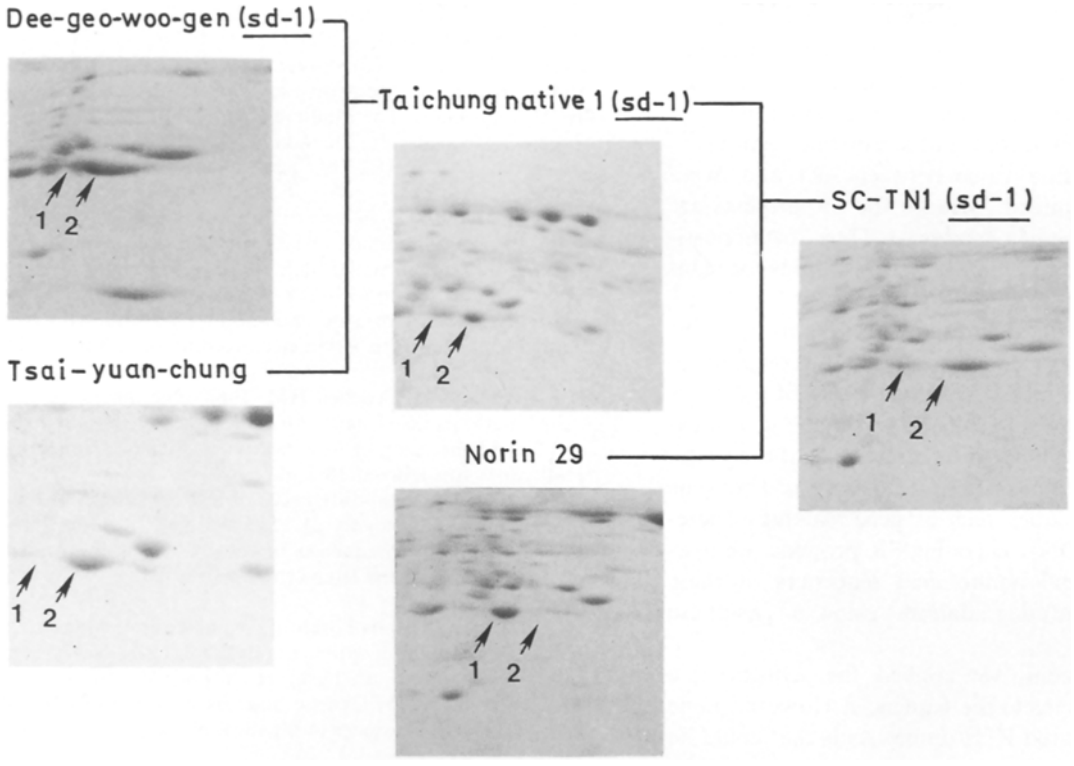


**Fig. 2.** Relation between the pedigree of SC-TN1 and SRP-2. Numerals 1 and 2 represent SRP-1 and SRP-2, respectively. SRP-2 was detected in all the rice cultivars and lines except for Norin 29. Detected by Coomassie blue staining

**Table 2.** Segregation of SRP-1 and SRP-2 proteins in rice embryo

| Population | Number of plants | | | Total | $\chi^2$ (1:2:1) | *P* value |
|---|---|---|---|---|---|---|
| | SRP-1 | SRP-1/ SRP-2 | SRP-2 | | | |
| Norin 29/ Norin 29 | 20 | 0 | 0 | 20 | | |
| SC-TN1/ SC-TN1 | 0 | 0 | 20 | 20 | | |
| SC-TN1/ Norin 29 $F_1$ | 0 | 20 | 0 | 20 | | |
| SC-TN1/ Norin 29 $F_2$ | 15 | 27 | 13 | 55 | 0.164 | 0.90–0.95 |

SRP-1 and SRP-2 were separated by 2D-PAGE and electroblotted onto a PVDF membrane. A total of 500 pmol–1 nmol of the blotted proteins was applied to the gas-phase protein sequencer to analyze the N-terminal amino acid sequences. However, no phenylthiohydantoin amino acid was released after ten cycles of Edman degradation.

## Discussion

Semidwarfism has contributed to the breeding of high-yielding rice cultivars (Futsuhara et al 1967; Chandler 1969; Chung and Heu 1980; Rutger 1983). A large number of genetic analyses has been performed in the semidwarfism of rice (Heu et al. 1968; Kinoshita and Shinbashi 1982). The semidwarf gene *sd-1* is one of the most valuable gene resources for breeding, because many semidwarf cultivars carry *sd-1* (Chang et al. 1985; Hu 1973; Mackill and Rutger 1979; Kikuchi et al. 1985), which is a recessive gene located on linkage group 1 (Suh and Heu 1978). Kikuchi et al. (1985) produced a semidwarf near-isogenic line that harbors *sd-1*, by four backcrosses of Norin 29 to TN1. In the present study, we used SC-TN1, which was produced by three further backcrosses of Norin 29 to the semidwarf near-isogenic line produced by Kikuchi et al. (1985). There was no apparent difference in the phenotypes of Norin 29 and SC-TN1, except for the semidwarfism. Therefore, SC-TN1 is considered to be one of the most suitable materials for the study of semidwarfism at the molecular level.

The current study revealed that Norin 29 and SC-TN1 differ in one protein. The different forms of this protein are under the control of two codominant alleles,

*Srp-1* and *Srp-2*, at the *Srp* locus. We also found that the semidwarf cultivars and SC-TN1, which have *sd-1*, also harbor *Srp-2*. However, a tall Taiwanese cultivar, Tsai-yuan-chung, also has *Srp-2* (Fig. 2), suggesting that *sd-1* and *Srp-2* are closely linked, but are distinct genes.

The recombination value between *sd-1* and *Srp-2* could not be determined, because the SR proteins are detected by using the whole embryo. Thus, it is impossible to determine the genotype of these embryos for plant stature.

If the amino acid sequence of SR proteins could be determined, it might be possible to synthesize oligonucleotides, which would be used for the cloning of the genes encoding the proteins as probes, and for the identification of the coding region and reading frame of the genes. The cloned genes might be used to identify *sd-1* by gene manipulation techniques such as gene walking. Therefore, to clone the DNA encoding SR proteins, we analyzed the N-terminal amino acid sequences of these proteins by Edman degradation, using a gas-phase protein sequencer.

In this experiment, we applied the sequenceable amount of the proteins to the sequencer. However, none of the proteins released PTH amino acids that could be identified after ten cycles of Edman degradation of the proteins, suggesting the presence of blocked N-terminals. It is concluded that to obtain information on the sequence of SR proteins, internal sequence analysis may be necessary.

## References


Aquino RC, Jennings PR (1966) Inheritance and significance of dwarfism in an Indica rice variety. Crop Sci 6:551–554

Athwal DS (1971) Semidwarf rice and wheat in global food needs. Q Rev Bol 46 (1):1–34

Chandler RF (1969) Improving the rice plant and its culture. Nature 221:1007–1010

Chang TT, Morishima H, Huang CS, Tagumpay T, Tateno K (1965) Genetic analysis of plant height, maturing and other quantitative traits in the cross of Peta × I-geo-tze. J Agric Assoc China News Serv 39:1–8

Chung GS, Heu MH (1980) Status of japonica-indica hybridization in Korea. In: Innovative approaches to rice breeding. IRRI, Los Banos, The Phillipines, pp 135–152

Futsuhara Y, Toriyama K, Tsunoda K (1967) Breeding of a new rice variety "REIMEI" by gamma-ray irradiation. Jpn J Breed 17:13–18

Gooderham K (1984) High-sensitivity silver staining of proteins following polyacrylamide gel electrophoresis. In: Walker JM (ed) Methods in molecular biology, vol 1, Proteins. Humana Press, Clifton, NJ pp 113–118

Hargrove TR (1979) Diffusion and adoption of semidwarf rice cultivars as parents in Asian rice breeding programs. Crop Sci 19:571–574

Heu MH, Chang TT, Beachell HM (1968) The inheritance of culm length, panicle length, duration to heading, and bacterial leaf blight reaction in a rice cross: Sigadis × Taichung (Native) 1. Jpn J Breed 18:7–11

Hirano H (1982) Varietal differences of leaf protein profiles in mulberry. Phytochemistry 21:1513–1518

Hirano H (1989) Microsequence analysis of winged bean seed proteins electroblotted from two-dimensional gel. J Protein Chem 8:115–130

Hu CH (1973) Evaluation of breeding semidwarf rice by induced mutation and hybridization. Euphytica 22:562–574

Kikuchi F, Itakura N, Ikehashi H, Yokoo M, Nakane A, Maruyama K (1985) Genetic analysis of semidwarfism in high-yielding rice varieties in Japan. Bull Natl Inst Agric Sci D 36:125-143

Kijimoto-Ochiai S, Katagiri YU, Ochiai H (1985) Analysis of N-linked oligosaccharide chains of glycoproteins on nitrocellulose sheets using lectin-peroxidase reagents. Anal Biochem 147:222–229

Kinoshita T, Shinbashi N (1982) Identification of dwarf genes and their character expression in the isogenic background. Jpn J Breed 32:219–231

Mackill DJ, Rutger JN (1979) The inheritance of induced-mutant semidwarfing genes in rice. J Hered 70:335–341

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Rutger JN (1983) Applications of induced and spontaneous mutation in rice breeding and genetics. Adv Agron 36:383–413

Suh HS, Heu MH (1978) The segregation mode of plant height in the cross of rice varieties. VI. Linkage analysis of the semidwarfness of the rice variety "Tongil." Korean J Breed 10:1–6