Glutinous (Waxy) Endosperm Starch Mutant of *Triticum monococcum* L.

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Cultivars or mutant lines which contain glutinous endosperm starch have been developed in seven crops, including rice, maize, barley, etc. However, reports on *Triticum* species with glutinous endosperm are not available. Attempts were made to induce the mutation of endosperm glutinousness in *Triticum monococcum* L. by treatment with EMS. Four hundred seeds of *T. monococcum* were pre-soaked for 20 h and treated with 0.4% (v/v) EMS for 24 h at room temperature. Germination percentage was 42.0%. After transplanting of the M₁ seedlings to a field, 99 M₁ plants died partly due to late transplanting and severe winter weather conditions. However, 2,016 M₂ seeds were harvested from the fertile 59 M₁ plants which survived. By staining the endosperm starch of the distal part of the seeds with a KI-I₂ solution, six out of 2,016 seeds showed a red brown color, which is the typical reaction of glutinous endosperm starch to KI-I₂. Consequently, these six M₂ seeds were considered to be glutinous endosperm mutants. The appearance of these seeds was not different from that of the seeds of normal *T. monococcum*. Two M₂ seeds were also obtained, in which the endosperm starch showed a reddish purple color rather than the typical purple color of non-glutinous starch. The present results indicate that glutinous endosperm mutants can be obtained in *Triticum* species.

KEY WORDS : *Triticum monococcum* L., cereal, EMS, mutant, glutinous endosperm, waxy starch.

Introduction

The endosperm starch of major cereal seeds is composed of about 20% amylose and 80% amylopectin. This type of endosperm starch is called "non-glutinous" or "non-waxy". On the other hand, the endosperm starch of glutinous starch cultivars, called "glutinous" or "waxy", is composed only or predominantly of amylopectin. When cooked, gelatinized glutinous starch is sticky and soft in texture and also resistant to the degradation of physical characters. These glutinous starch characteristics have been utilized in East Asian traditional foods and recently in food processing industries.

Cultivars with glutinous endosperm have been reported in seven crop species, including rice, maize, barley, Job's tears, foxtail millet, common millet and sorghum (SAKAMOTO 1982). In rice and maize, several endosperm starch mutants such as amylose extender (*ae*), dull (*du*) or sugary (*su*) have also been obtained artificially (CREECH 1968, SATOH and OMURA 1981). However, no varieties or mutants with glutinous endosperm starch have been reported in *Triticum* species.

The present study was carried out to determine whether it is possible to obtain mutants of glutinous endosperm starch in *Triticum* species, by treating diploid *T. monococcum* with EMS.

Materials and Methods

The seeds of *Triticum monococcum* L. line No. KT 3-5, which was derived from early maturing mutants of *T. monococcum*, were pre-soaked in distilled water for 20 h at room temperature. Thereafter, the seeds were treated with 0.4% (v/v) EMS (Ethyl Methanesulfonate, Eastman Kodak Co.) for 24 h at room temperature. Subsequently, they were washed thoroughly with running water for 1 h, then sown on seedbeds in a green house. After 4 weeks, the M₁ seedlings were transplanted to a field. At the flowering stage, the pollen of these plants was examined using a KI-I₂ solution (final concentration, 0.2% (w/v) KI and 0.04% (w/v) I₂) to differentiate the pollen of glutinous starch plants from that of non-glutinous starch ones. At harvest, each spike of the plants was collected separately and numbered. Seed fertility was estimated as the percentage of seeds in about fifteen of the first florets in the middle part of each spike.

Glutinousness of the endosperm was also examined by staining the crushed endosperm of the distal part of the seeds with the KI-I₂ solution. The remaining halves of the seeds with the embryo were stored in a refrigerator. The color of the endosperm starch stained with the KI-I₂ solution was observed under a microscope.

Results and Discussion

Four hundred seeds of *T. monococcum* line No. KT 3-5 were treated with EMS (Table 1). No. of seeds which germinated was 168 and the germination percentage was 42.0%. After transplanting of the seedlings to a field at the third or fourth leaf stage, 99 seedlings withered, partly due to late transplanting and unfavorable winter weather conditions. Sixty nine plants survived, of which ten plants failed to set seed at harvest time. A number of 439 spikes were collected from 59 plants. There were 198 fertile spikes and each of them was numbered. The percent of fertile spikes was 45.1% (198/439), and 2,016 seeds were obtained from these spikes. The seed fertility of the spikes was 42.6% on the average, which was significantly lower than that of the normal *T. monococcum* line No. KT 3-5 (98.0%).

Treatments of other crops with EMS have been reported including rice (pre-soaking for 60 h, 0.002% EMS for 4 h, YAMAGUCHI 1972, pre-soaking for 4 h, 0.03-0.2% EMS for 7 h, FUJIMOTO and YAMAGATA 1982), barley (pre-soaking for 24 h, 0.26% EMS for 24 h, EHRENBERG *et al.* 1966), and common wheat (pre-soaking for 8 h, 0.35% EMS for 16 h, FRANCKOWIAK *et al.* 1976). Compared with these findings, the current EMS treatment applied to *T. monococcum* was drastic and resulted in a low level of germination, viability and fertility of the M₁ plants.

Table 1. EMS treatment and seed fertility of M₁ plants.

No. of seeds treated with EMS	No. of seedlings	No. of plants survived	No. of spikes harvested			No. of M ₂ seeds collected
			Total	fertile	sterile	
400	168	69	439	198	241	2,016
(100)*	(42.0)	(17.3)	(100)	(45.1)	(54.9)	

* : percent.

Table 2. Screening M₂ seeds for waxy endosperm starch by KI-I₂ staining.

Entry	No. of examined	Color of starch granules		
		Purple	Red-brown	Reddish purple
Spikes	198	198	2	2
Seeds	2,016	2,008	6	2

It is well known that glutinous starch shows red brown color, while non-glutinous starch is blue or purple when they are stained with KI-I₂ solutions. The endosperm starch of the distal half of 2,016 seeds was examined using the KI-I₂ solution. Six out of ten kernels of two spikes (spike Nos. 55-1 and 55-2) showed red brown starch when stained with KI-I₂ (Table 2, Fig.1 a). The large starch granules stained light red brown at the periphery and dark red brown at the core of the granule. The small granules did not exhibit darkly stained cores. On the other hand, the starch granules from the other M₂ seeds including the remaining four seeds of spike Nos. 55-1 and 55-2 and normal seeds of *T. monococcum* were purple (Fig.1 b). Consequently, these results indicated that six seeds from the two spikes (Nos.55-1 and 55-2) were mutants with glutinous endosperm starch.

Endosperm starch of two seeds from other two spikes (spike No. 54 and 67-1) stained reddish purple. This suggested that these M₂ seeds may be heterozygotes of the normal non-glutinous (*Glt*) and mutant glutinous (*glt*) gene, and that the endosperm genotype is *Glt*, *Glt*, *glt* or *Glt*, *glt*, *glt* as observed in rice (SANO 1984). It is also possible that these seeds were endosperm mutants as in the case of the "low amylose" or "dull" ones in rice or maize (OKUNO 1983).

Seed fertility of the four spikes (spike Nos. 55-1, 55-2, 54 and 67-1), which was 5.9, 33.3, 10.0 and 18.8% respectively, was lower than the average of the spikes of M₁ plants (42.6%). These results suggest that the primordial cells of these spikes were markedly affected by the EMS treatment and that the mutation for the glutinous character was induced by a relatively substantial genetic change.

The appearance of seeds is known to be different between glutinous and non-glutinous cultivars in several crops. In rice, the non-glutinous seeds are more translucent than the glutinous seeds. The mature seeds of several cultivars of glutinous *H. vulgare* show a purple color due to the accumulation of anthocyanine on the seed coats (NAKAO 1950). However, the seeds of the present *T. monococcum* glutinous mutant were not different in appearance from the normal seed (Fig.2).

Normal *T. monococcum* endosperm starch was separated into large granules (ca. 30 micron diameter) and small granules (a few micron diameter), as in the case of *T. aestivum* (DENGATE 1983). The starch granules of the glutinous mutant of *T. monococcum* were not different in shape and size from those of normal *T. monococcum*. The present glutinous mutations appear not to affect morphological characteristics of the starch granules of *T. monococcum*.

At the flowering time of the M₁ plants, the pollen of each plant was examined using the KI-I₂ solution. In the anthers of four M₁ plants, a mixture of pollens staining red brown and purple was observed. The endosperm starch of these plants was re-examined

using the KI-I₂ solution. However, no M₂ seeds with glutinous endosperm starch were detected. At present, the efficiency of the KI-I₂ staining for pollen starch to identify glutinous endosperm starch mutants remains to be elucidated.

Further analyses of the *T. monococcum* glutinous mutant will be conducted in the next generation.

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Fig. 1 Endosperm starch granules of *T. monococcum* seeds stained with KI-I₂ solution.

- a, Spike No. 55-1 (red-brown). Bar = 100 μm
- b, Normal *T. monococcum*. Bar = 100 μm

Fig. 2 Appearances of *T. monococcum* seeds.

- a, Non-glutinous starch M₂ seeds of spike No. 30 .
- b, Glutinous starch M₂ seeds of spike No. 55-1 .
- c, Normal seeds.

一粒系コムギにおけるモチ性胚乳デンプン突然変異

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イネ科穀類の種子胚乳デンプンは通常はウルチ性であるが, イネ, オオムギ, トウモロコシ等の7種にはさらにモチ性品種が存在する. しかしコムギ属 (*Triticum*) にはモチ性デンプンを持つ品種, あるいは突然変異系統の報告は未だされていない. 我々は二倍性コムギである一粒系コムギ (*Triticum monococcum* L.) に EMS による人為突然変異を誘発し, その後代からモチ性変異株を得ようと試みた. EMS 処理は室温で予備吸水 20 時間の後, 濃度 0.4% (v/v) で 24 時間行った. 処理後発芽した幼苗を畑に移植して栽培, 収穫した. 処理を行った 400 粒の発芽率は 42.0% で, その後生存し種子をつけた植物は, 処理した種子の 14.7% であった. また着粒した穂数及び種子粒数は 198 穂, 2,016 粒でそれらの穂の中央部約 15 の第一小花における種子稔性は 42.6% であった (Table 1). これらの結果は, M_1 幼苗移植が遅れ冬がきびしかったことを考慮しても, 今回の EMS 処理が *T. monococcum* にはきつかったことを示していると思われる. 得られた種子は胚を含まない半粒部をつぶして KI- I_2 溶液を滴下し顕微鏡下で胚乳デンプンの色を観察した. その結果, 2 穂からの 10 粒のうち 6 粒の胚乳デンプンが赤褐色に染まった (Table 2, Fig. 1 a). 他の粒は総て青紫色であった (Fig. 1 b). このことから胚乳デンプンが KI- I_2 溶液で赤褐色に染まる M_2 種子はモチ性の突然変異であると思われる. これらの種子はイネで見られるような胚乳部の透過程度の違いや, 「オオムギモチ種のように種皮がアントシアニンで紫色になることはなく, 外観上は通常のウルチ性 *T. monococcum* 種子と変わらなかった (Fig. 2). またデンプン粒の大きさ形状でも正常な一粒系コムギとの間に違いはみられなかった. さらに他の 2 穂からの 2 粒は, 胚乳デンプンが赤紫色に染まった. これらはモチ遺伝子に関する異型接合体か或は「低アミロース」, 「ダル」のような突然変異かも知れないが今後の分析が必要である. これらの胚乳突然変異と思われる M_2 種子をつけた M_1 穂の種子稔性は 5.9~33.3% で, M_1 の平均 42.6% より低く, EMS による障害を強く受けた細胞から出現したと思われる. M_1 植物が開花したとき, 各個体からの穂の花粉を KI- I_2 溶液で染めて観察した. 4 個体からの 6 穂の蒴の花粉の一部が赤褐色に染まった. しかしそれらから得られた M_2 種子の胚乳デンプンはすべてウルチであった. 得られたモチ性突然変異については現在後代を育成中であり, 今後さらに遺伝的分析を行う予定である.