Varietal Difference of Plant Regeneration from Root Callus Tissues in Rice

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Tissue cultures were initiated from root sections excised from 5~7 day-old seedling in 60 varieties including various ecospecies i. e. *japonica*, *indica*, *japonica-indica* hybrids, and large grain varieties (assumed to be *javanica* type) in rice (*Oryza sativa* L.) on basal Murashige and Skoog's (1962) medium containing 3.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 2.0 g/l of casein hydrolysate, and 30 g/l sucrose. Cultures were maintained on the same medium with 2.0 mg/l 2,4-D. Plant regeneration was accomplished by subculturing for 2 to 3 passages to the same basal medium with 0.02 mg/l 2,4-D and 10 mg/l kinetin. Remarkable variability in plant regeneration was observed among varieties tested. All the cultures of the *japonica* varieties had good capacity for regeneration, while some of the cultures of *indica*, *japonica-indica* hybrids, and large grain varieties were poor. It was observed that 11 of 28 cultures in *japonica*, 2 of 16 in *indica*, and 1 of 10 in large grain varieties had relatively high potential for regeneration at more than 30%. Besides normal plant regeneration, cultures showed various responses such as massive rooting without shoot formation, retention of green pigmented region without further development, and occurrence of necrotic dead tissue. Two distinct types of plant regeneration were observed, one of which was organogenesis, and other was somatic embryogenesis. Most of the cultures from the *indica* varieties, Chyokoto and Gaiya Dhan Tosar, initiated plantlet through somatic embryogenesis.

KEY WORDS : *Oryza sativa* L., plant regeneration, organogenesis, embryogenesis, genotypic difference, root callus tissue, tissue culture.

Introduction

There are many reports on *in-vitro* plant regeneration in rice. Among them first reports were by NISHI *et al* (1968) using seed, NIIZEKI and OONO (1968) using anther, and KAWATA and ISHIHARA (1968) using root cultures. Subsequent reports were on successful plant regenerations from immature embryos (LAI and LIU, 1982) and leaf tissues (HENKE *et al.*, 1978; WERNICKE *et al.*, 1981). Rice plant regeneration conducted with special reference to the effect of medium composition, such as phytohormones, were carried out by SAKA and MAEDA 1966; NISHI *et al.*, 1973; HENKE *et al.*, 1978; INOUE and MAEDA, 1980. Other works were related to the changes of hydrolytic enzymes (SAKA and MAEDA, 1974) and histology (NAKANO and MAEDA, 1979). In anther culture, various mutants (e. g. polyploidy, dwarf, and partial sterile plants) had been recovered (NISHI and MITSUOKA 1969; OONO 1975). Recent report indicated that mutants could also be obtained from plantlets regenerated from seed cultures (FUKUI, 1983). Plant regeneration through embryogenesis were also reported from anther (GENOVESI and MAGILL, 1982), immature embryo and seed cultures (HEYSEY *et al.*, 1983).

The influence of genotype on the cereal plant regeneration had been reported on maize (GREEN and PHILLIPS, 1975), oat (CUMMINGS *et al.*, 1976; RINES and MCCOY, 1981), barley (FOROUGH-WEHR *et al.* 1982), wheat (SEARS and DECKARD, 1982; AHLOOWALIA, 1982). Similar observation in rice were reported on anther cultures (GUHA-MUKHERJEE,

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1973; WAKASA 1981), and seed cultures (TAMURA, 1981). However, only a small number of genotypes have been investigated. The objective of this study is to investigate the influence of varietal difference on the capacity for plant regeneration, using root explants, and to possibly identify varieties that can be utilized for such studies as cell and protoplast cultures.

Materials and Methods

Sixty varieties, including *japonica*, *indica*, *japonica-indica* hybrids and large grain varieties assumed to be *javanica* types, were used for the investigation. Twenty five kernels taken from dehusked mature seed were selected from each of the varieties. Seeds were sterilized, using 70% (v/v) ethanol for about 30 sec. and 1% (v/v) sodium hypochlorite for 40 min. and subsequently rinsed with sterilized distilled water 3 times in 1×6 cm petri dish. Petri dishes containing seeds and about 3 ml sterilized water sealed with 'Parafilm' were maintained at 26°C under about 1500 lux light intensity from cool-white fluorescent lamp. After 5~7 days of incubation, the initiated and elongated seminal and some nodal roots were excised to 1 cm length for inoculation.

Basal medium used was Murashige and Skoog's (1962), supplemented with 1 mg/l thiamin-HCl, 200 mg/l mio-inositol, 3 mg/l 2,4-D, 2 g/l casein hydrolysate, 30 g/l sucrose and 8 g/l agar. The medium was adjusted to pH 5.8, and 50 ml of medium solution was poured into each of the 100 ml flasks and autoclaved at 1.2 kg/cm² for 15 min. About 20 root sections, excluding the terminal root tips, were placed on the medium surface of each of the flasks. Two flasks were used for each of the varieties. The cultures were maintained under the same conditions of temperature and light as those for germination. After 40 days incubation, only the callus tissues initiated were subcultured to similar medium except with reduced 2,4-D concentration at 2 mg/l. Plant regenerations from the second and third passages of callus cultures were placed on the regeneration medium having similar constituents as the callus initiation medium, except that the 2,4-D concentration was reduced to 0.02 mg/l while 10 mg/l kinetin was added. All cultures were kept at 26°C under continuous light of about 3000 lux intensity from cool-white fluorescent lamp. After 30 days incubation, the number of cultures differentiating into shoots, roots, and having green pigmented spots were assessed.

Results

When 1 cm of root sections were inoculated onto the callus initiation medium containing 3.0 mg/l 2,4-D, it was generally observed that lateral root tips changed to small globular callus tissues after 10 days of incubation. Frequencies of callus initiation from root sections were about 70 to 100%, although they varied according to the varieties. The callus tissues initiated were highly either mucilaginous and translucent or covered with thick membrane of sticky substances in early stage of culture, and developed compact pale-yellow callus consisted of many nodular tissues in late stage of culture. After the first subculture, the callus tissues grew to about 5 mm diameter of masses with many small globular structures on the surface. Occasionally, numerous root-like structures without the response to the gravity initiated from the callus surface, although the frequencies of initiation of these structures varied according to the varieties. In general,

Fig.1-15. Various aspects of plant regeneration.

Fig.1. Plantlet with shoot and root formations in Rikuu 132, $\times 3$.

Fig.2. Only shoot differentiating without root in Rikuu 132, $\times 3$.

Fig.3. Plantlet with shoots, roots and callus tissue in Murasaki-ine, $\times 3$.

Fig.4. A differentiated root from callus tissue in Murasaki-ine, $\times 3$.

Fig.5. Differentiated shoot and Pin-head-like green spot in Hoyoku, $\times 3$.

Fig.6. A mass of plantlets consisted of about 20 individuals differentiated from one cultures in Hoyoku, $\times 0.6$.

Fig.7. Regenerated callus with green pigmented regions in Joshu, $\times 3$.

Fig.8. Many plantlets with shoot and root which were developed from embryoids in Gaiya Dhan Tosar, $\times 3$.

Fig.9. Nine plantlets regenerated from one cultures in Gaiya Dhan Tosar, $\times 0.6$.

Fig. 10. Three plantlets with shoot and root which were developed from embryoids in Amber, $\times 3$.

Fig. 11. Albino plants initiated among green plants in Te-Tep, $\times 3$.

Fig. 12. Proliferated plantlets with more than 10 shoots and 6 roots in Anthocyane, $\times 3$.

Fig. 13. Only shoots differentiated from callus tissue in Te-Tep, $\times 3$.

Fig. 14. Numerous root-like structures and fine root hairs on the root-like structures differentiated from culture surface in Zenith, $\times 3$.

Fig. 15. Plant regeneration from five cultures in a flask in Gaiya Dhan Tosar, $\times 0.5$.

Fig. 16. Severe necrotic dead cultures in British Honduras Creole, $\times 0.5$.

In Fig. 1, 2, 3, 4, 5, 8, 10, 11, 12, and 13, regeneration were observed 20 days after incubation. In Fig. 6, 7, 9, and 14 regeneration were observed 30 days after incubation, and Fig. 15 and 16 were cultures after 40 days incubation. Abbreviation, AL : abnormal robed leaf, AP : albino plant, C : callus tissue, CL : coleoptile, GR : green pigmented region, FL : first leaf, NC : necrotic dead callus, R : root, RH : root fine hair, RS : root-like structure, S : shoot.

calluses of *japonica* showed better growth than those of *indica* and large grain varieties. *japonica* calluses lost the mucilaginous trait in the early stage, while *indica* and large grain varieties maintained this trait over a large period.

When transferred to the regeneration medium, callus tissues inoculated became partial brownish in almost all the varieties. Some callus tissues were more susceptible to browning and became severely necrotic. Others appeared to be tolerant to browning and necrosis, and grew, initiating more callus. About 7 to 10 days after subculture, green pigmented spots which will develop into shoot thereafter were observed on the tissue surfaces (Fig. 7). After about 20 days, plantlets were initiated from original callus tissues. Coleoptile grew to about 1 cm long and first leaf emerged from top of the coleoptile (Fig. 2, 8, 10, 12). Occasionally, abnormal lobed leafy structures were seen at the base of the shoot (Fig. 1, 3, 5). After 30 days, second and third leaves emerged, and the plantlets grew to about 5 cm height with the roots attached to the base of the shoot (Fig. 6, 9). Allowing further development of the regenerated plants in the flask for 40 days, leaves of the regenerated plants attained to top of the flask, but roots which developed downward into the agar became gradually brownish (Fig. 15).

Two distinct types of plant regeneration were observed. One of these was organogenesis; the tiny green shoots were first initiated on the callus surface, and roots were subsequently initiated at the base of the shoot, or occasionally there was not root initiation (Fig. 2, 5, 13). In a few cases, only roots were initiated and developed without shoots or shoot primordia (Fig. 4). This type of plant regeneration was observed comparatively in many *japonica* and some *indica* varieties (e. g. Te-Tep, Fig. 13). The other was somatic embryogenesis; embryo-like bodies surrounded by scutellum-like structures were first formed and then grew into plantlets with bipolar structure which had both shoot and root, and with shoot-root association (Fig. 1, 3, 8, 10, 12). The typical examples of this plant regeneration type were observed in *indica* (e. g. Chyokoto, Gaiya Dhan Tosar, and Amber) and in some *japonica* and large grain varieties. There were some cultures exhibiting both types of differentiation. Especially, these cultures could be seen in *japonica* varieties. In organogenesis, some albino plants were observed, and occasionally, both green and albino plants were derived from the same callus tissue (Fig. 11). The plantlets induced by embryogenesis were phenotypically similar to seedlings raised normally from seeds. Fig. 9 shows 9 plantlets regenerated from similar callus tissue after 30 days incubation. These showed wide variation in plant growth ranging from small plantlet (about 0.5 cm shoot length) to large one (about 8 cm shoot length).

Difference was observed among the varieties in their capacity to regenerate plants. Table 1 shows the frequency of cultures initiating shoots, green pigmented spots, and roots in second and third passages of subcultures. In general, the frequencies of cultures capable of initiating plantlets or green pigmented spots were higher in the tissues from passage III than those from passage II. Comparing the capacity to regenerate among the varieties in both cultures from passages II and III, all the cultures of *japonica* varieties showed higher regenerative capacity while some of the cultures of *indica*, *japonica-indica* hybrids, and large grain varieties showed poor capacity to regenerate plants.

Japonica varieties, Rikuu 132, Reiho, and Murasaki-ine showed high regenerative

Table 1. Frequency of plant regeneration from callus tissues derived from root sections in rice.

Varieties	No. of cultures initiating shoot		No. of cultures initiating green spot		No. of cultures initiating shoot or green spot		No. of cultures initiating root	
	Passage II	III	Passage II	III	Passage II	III	Passage II	III
<i>japonica</i>								
Joshu	3	3	0	12	3	15	4	19
Sen-ich	1(1)	3(3)	1	0	2(1)	3(3)	2	6
Aikoku	4	5(4)	0	4	4	9(4)	4	10
Kamenoo	1	5(2)	1	4	2	9(2)	1	7
Asahi	1	2(1)	0	0	1	2(1)	1	4
Rikuu 132	6(2)	7(1)	2	6	8(2)	13(1)	8	15
Norin 1	0	2	1	0	1	2	1	4
Norin 22	1	2	1	0	2	2	3	6
Ishikari	2	1	0	7	2	8	2	9
Fujisaka 5	5(2)	4(1)	2	3	7(2)	7(1)	9	14
Fujiminori	6	3(1)	1	2	7	5(1)	5	5
Sasanishiki	5	6(5)	0	3	5	9(5)	5	13
Koshihikari	2(1)	6(3)	3	1	5(1)	7(3)	6	6
Nipponbare	4(1)	6(1)	0	4	4(1)	10(1)	7	11
Kinmaze	1(1)	0	0	0	1(1)	0	1	0
Hoyoku	6(1)	4(1)	3	2	9(1)	6(1)	8	8
Reiho	4	15(5)	1	0	5	15(5)	5	16
Kuju	2(2)	1(1)	0	0	2(2)	1(1)	3	2
Nakate-shinsenbon	1	2	0	0	1	2	1	2
Tamanishiki	1	3(1)	1	4	2	7(1)	2	14
Somewake	2	3(3)	0	2	2	5(3)	3	6
Murasaki-ine	5	9(1)	2	0	7	9(1)	1	9
Taichung 65	1	1(1)	1	1	2	2(1)	3	4
Jaekoon	2	0	0	0	2	2	2	2
Calrose	-	0	-	9	-	9	-	9
Reimei	1	2	1	1	2	3	4	12
Tan-ginbozu	5(2)	2(1)	0	0	5(2)	2(1)	6	4
Daikoku 1	6	0	0	6	6	6	5	6
<i>japonica</i> × <i>indica</i>								
Tongil	0	0	0	3	0	3	0	5
Josaeng Tongil	0	0	0	1	0	1	0	8
Milyang 21	0	0	0	2	0	2	0	0
Milyang 23	-	0	-	0	-	0	-	0
Suweon 256	0	0	0	0	0	0	0	0
Yushin	0	2(2)	0	0	0	2(2)	0	9
<i>indica</i>								
Amber	2	1	0	1	2	2	1	1
British Honduras Creole	0	0	0	0	0	0	0	0
Blue Belle	0	0	0	4	0	4	0	14
Century Patna	0	0	0	3	0	3	0	2
Chyokoto	9	5	2	2	11	7	7	16
Gaiya Dhan Tosar	9	14	2	0	11	14	3	14
Kannonsen	0	0	0	0	0	0	0	0

Varieties	No. of cultures initiating shoot		No. of cultures initiating green spot		No. of cultures initiating shoot or green spot		No. of cultures initiating root	
	Passage II	III	Passage II	III	Passage II	III	Passage II	III
Kinandang	0	0	0	0	0	0	0	0
Konansen	-	2	-	0	-	2	-	10
Hadsadri	0	0	0	1	0	1	0	1
IR-8	-	3	-	1	-	4	-	7
Mao-zu-tao	0	3(1)	1	0	1	3(1)	0	3
Panbila	-	0	-	0	-	0	-	0
Taichung Native 1	-	0	-	0	-	0	-	0
Te-Tep	-	18(8)	-	1	-	19(8)	-	19
Zenith	0	0	0	1	0	1	8	4
Large grain var.								
Allorio	2	1	9	12	11	13	6	12
Anthocyane	3	1	0	6	3	7	1	8
Arditane	-	1	-	0	-	1	-	1
Lady Wright	0	0	1	0	1	0	6	0
Lomelto	-	1	-	0	-	1	-	3
Rinnatto	-	0	-	3	-	3	-	8
Secia	-	0	-	2	-	2	-	3
Senatore	0	0	0	0	0	0	0	2
Stripe 136	0	1	0	0	0	1	0	2
S-82	-	1	-	0	-	1	-	3

In each variety, 20 cultures weighing 30~50mg were used for inoculation on Murashige Skoog's (1962) medium containing 0.02mg/l 2,4-D, 10mg/l kinetin, and 2g/l casein hydrolysate. Parenthesis shows the number of cultures inducing only albino plant regeneration.

capacity, their average frequencies being 32.5~42.5% in shoot initiation, 25.6~62.5% in root initiation, and 40.0~42.5% in regenerative culture initiation on cultures from passages II and III. Fujisaka 5, Fujiminori, Sasanishiki, Koshihikari, Nipponbare, and Hoyoku followed to the three former elite varieties with the respective frequencies of 20.0~27.5%, 25.0~62.5%, and 30.0~37.5%. In a variety, Joshu, most of the cultures from a passage III were regenerative, but only a few cultures initiated shoots and many cultures remained green pigmented spots without further development. Percentage of cultures initiating albino plants was higher in the cultures from passage III (37%) than from passage II (17%) on an average in all the cultures initiating shoots in *japonica* varieties. Eighty percent of var. Aikoku and eighty three percent of var. Sasanishiki showed differentiation of albino plant from passage III cultures.

In *indica* varieties, cultures from varieties, Chyokoto, Gaiya Dhan Tosar, and Te-Tep showed high capacity for plant regeneration. The average frequencies of the regeneration of Chyokoto and Gaiya Dhan Tosar were 35.7 and 57.5% in shoot initiation, 42.5~57.5% in root initiation, and 45.0~62.5% in regenerative culture initiation, respectively, from passages II and III. Regeneration in these two varieties showed typical somatic embryogenesis-like differentiation. On the other hand, the regeneration type of the cultures in Te-Tep appeared to be organogenesis (Fig.11,13), with its frequencies being very high, 90% in shoot initiation, 95% in root initiation, and 95% in regenerative culture

initiation in the cultures only from passage III. In addition, the percentage of the cultures differentiated only albino plants was relatively high (44%) in Te-Tep. In Blue Belle, Konansen, and Zenith, the cultures showed massive rooting without shoot and shoot primordium formations. The cultures of Zenith from passage II produced many root-like structures with fine root hairs radiating outwards (Fig. 14).

A large grain varieties, Allorio, showed good response to regenerative culture initiation, although the frequency of cultures differentiated into shoots was relatively low. Many cultures retained green pigmented spots without further development as observed in the cultures of a *japonica* variety, Joshu. In *japonica-indica* hybrids, which are mainly cultivated in Korea, the frequencies of cultures showing differentiation were very low in cultures from passage II and III.

Generally, in all varieties, most of cultures showed high capacity for plant regeneration, maintained growth despite necrosis, but other cultures showing no plant regeneration were liable to be susceptible to browning and necrosis and consequently died off the medium containing reduced 2,4-D and high concentration of kinetin (Fig. 16).

Discussion

It was seen that the root callus tissues were highly mucilaginous in the early initiation stage, and numerous root-like structures were initiated in the subculture stage, although these traits varied largely among varieties. It has been shown that root callus tissues were derived from lateral root primordia (NISHIMURA and MAEDA, 1983), and seed callus tissues were derived from epithelium of scutellum (NISHIMURA and MAEDA, 1977) in rice. It appears that morphological traits of rice callus tissues vary due to their origins. Morphological variations were detectable for this subculture period. The results could suggest that endogenous phytohormone contents and their balance may vary between the callus tissues derived from root and scutellum.

The strong influence of genotype on plant regeneration has been observed in cereals as mentioned above. In our investigation, remarkable differences in response were observed in several rice varieties, including *japonica*, *indica*, *japonica-indica* hybrids and large grain types, some of showing high capacity for plant regeneration, while others do not. The *japonica* varieties, Rikuu 132, Fujisaka 5, Fujiminori, Sasanishiki, and Koshihikari, which show high regenerative capacity were closely related from the genealogical standpoint. Fujisaka 5 is a progenitor of Fujiminori, while Hoyoku is also a progenitor of Reiho. It could be possible that such trait as the high responsiveness of parents for plant regeneration might be transferred to their progeny. On the other hand, the cultures of Norin 1 showed low capacity for plant regeneration, although the cultures of Rikuu 132, parent of Norin 1, showed high capacity for plant regeneration. From these result, it may be assumed that the cultures of another parent of Norin 1 have low plant regeneration potential and it low responsiveness transferred to the progeny, Norin 1. Some of the cultures showed low responsiveness, while many cultures became seriously necrotic and died on the medium with reduced concentration of 2,4-D (0.02 mg/l) and high concentration of Kinetin (10 mg/l) during 30 day incubation. This appeared to be prominent among many *indica* varieties and *japonica-indica* hybrids. Among these

indica varieties, Taichung Native 1 and IR-8 which are both progenitor of the hybrids, showed similar responsiveness to the hybrids, although IR-8 had slightly higher responsiveness than Taichung Native 1 and the hybrids. These results could suggest that the capacity for plant regeneration is inherited and is probably under the genetic control as indicated by FOROUGHI-WEHR *et al.* (1982) with barley anther culture.

Variation in the rice seed callus formation attributed to varieties has been already reported (MAEDA, 1967; ABE and SASAHARA, 1982). In this experiment, though the ability to form callus tissues derived from rice root and that of callus growth were scored visually, varietal differences on callus formation and growth were similarly observed. The capacity for callus formation, growth, and plant regeneration in Rikuu 132, Fujisaka 5, Fujiminori, Sasanishiki, Koshihikari, Nipponbare, Hoyoku, Reiho, Murasaki-ine, Chyokoto, Te-Tep and Allorio were high. However, callus formation and growth in Asahi, Jaekeon, Kuju and British Honduras Creole were high, while the plant regeneration in these cultures was poor. On the other hand, callus formation and growth in Gaiya Dhan Tosar was relatively low, while the cultures showed high capacity to regenerate plants. Thus, it is difficult to indicate a close correlation between capacity of callus formation or growth and plant regeneration in rice cultures.

Rice varieties are known to differ in contents of phytohormones during seed maturation (ORITANI *et al.* 1983). The different response of cultures in plant regeneration among varieties may thus be due to the influence of endogenous phytohormone concentration. The cultures exhibiting high frequency of plant regeneration could have appropriate endogenous phytohormonal content as suggested in barley anther culture (FOROUGHI-FRIEDT, 1982) and in wheat embryo culture (AHLOOWALIA, 1982).

As mentioned above, two different types of plant regeneration were observed, one of these is organogenesis which had been commonly reported in rice (KAWATA and ISHIHARA, 1968; SAKA and MAEDA, 1969; HENKE *et al.* 1978), and the other is somatic embryogenesis which had been reported in only a very few reports in rice (GENOVESI and MAGILL, 1982; HEYSER *et al.*, 1983). With the somatic embryogenesis observed in this experiment, original embryoids which were enclosed by scutellum-like structure first formed and then grew into plantlets with bipolar structure as similarly reported in pearl millet (VASIL and VASIL, 1981). This phenomenon observed in several *indica* varieties e.g. Chyokoto, Gaiya Dhan Tosar, and Amber appears to be suitable for plant regeneration scheme and whole plant development, because numerous plants can be recovered from a small clumps of cultures. Thus this type of plant regeneration may also be favorable due to genetic stability. Histological observation on the rice embryoid through somatic embryogenesis is currently being looked into. This investigation should provide information on the genotypic differences relating to the capacity for plant regeneration in rice varieties of *japonica*, *indica*, *japonica-indica* hybrids, and the large grain varieties. The varieties exhibiting high capacity for plant regeneration can thus be exploited for the crop improvement through tissue culture in the future. Plant regeneration from a single cell has not yet succeeded in rice, although a few successful reports concerning rice protoplast culture has been published (DEKA and SEN, 1976; NIIZEKI and KIKI, 1981). We hope that these varieties can be utilized for studies relating to single cell, suspension

and protoplast cultures.

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イネの根由来カルス組織からの器官再分化品種間差異

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日本型、インド型、日印雑種および大粒種を含む 60 イネ品種の根断片を用いて、3mg/l の 2,4-D を含む MS の寒天培地に置床し、カルスの誘導を行なった。さらにそれらを 2mg/l の 2,4-D を含む MS 培地で 1~2 回継代培養を行ないカルスを増殖させ、ついで 2,4-D を 0.02mg/l に減じ、新たに 10mg/l のカイネチンを加えた同様の MS 培地に移し、再分化を図った。その結果、再分化の頻度には著しい品種間差異が認められた。継代を異にするものからの再分化を総合して、日本型の品種はすべて再分化を示したのに対し、日本型以外の品種の中には、全く再分化を示さない品種もあった。また再分化の不良な品種のカルスは、再分化培地に置床すると、強い necrosis が生じて死滅するものが多かった。茎葉と、その原基と考えられる緑色を帯びた小突起の分化によって、再分化能力を持つカルス組織の割合をみると、日本型は 16 のうち 11、インド型は 16 のうち 3、大粒種は 10 のうち 1 品種が、それぞれ 30 % 以上の再分化能力を示した。日印雑種では、30% 以上の再分化能力を示したものは全くなかった。日本型品種の中には、藤 5 坂号とフジミノリ、ハウヨクとレイハウのように親子関係にあるものが共に高い再分化能力を示すものがあった。さらにインド型の台中在来 1 号と、その後代系統である日印雑種の再分化能力は共に低く、一般に強い necrosis が観察された。これらのことから、イネの再分化能力は後代に伝わり、一定の遺伝的支配を受けているのではないかと考えられる。さらにイネの根から形成されたカルスは、品種によって形態や再分化培地に置床した場合の反応性が異なることから、内性ホルモンの含量やバランスも品種や生態種によって異なることが考えられる。

2 種類の異なった再分化様式が観察された。1 つは従来から観察されていた、不定芽や不定根を分化する不定器官形成であり、もう一つは、幼芽と幼根を持つ二極性の不定胚ができて、それが発達し、植物体となる不定胚形成である。日本型の品種では、一般に不定器官形成が見られたが、2 つの分化様式が同一品種内においても混在していたり、明確に識別できないこともあった。インド型と大粒種のうち数品種では典型的な不定胚形成とその発達が認められた。特にインド型の 2 品種では、この様式により高頻度で植物体を再生した。

茎葉と根を形成する正常な分化の他に、緑色を帯びて分化の初期の徴候を示しているのに、茎葉や根が形成されなかったもの、茎葉を分化することなしに、根だけ旺盛に分化したもの、さらにカルス組織の表面から多くの根様構造物を放射状に分化したものなどが品種によっては観察された。