

Genotypic differences in morphology and ultrastructures of callus derived from selected rice varieties

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The genotypic differences of callus cultures derived from selected rice varieties belonging to three subspecies namely *Indica*, *Japonica* and *Javanica* were evaluated based on their morphology and ultrastructures. Genotypic differences were observed in the morphology as well as in the ultrastructures of the selected varieties under each subspecies. Based on morphology, two types of calli were observed two weeks after culture in the *Indica* varieties: one was light yellow, compact, smooth-surfaced and the other was yellowish with hair-like projections and tiny green spots. The calli of the *Japonica* and *Javanica* varieties were yellowish, compact and had smooth surface. Four weeks after culture, progression of the green spots was noted in the *Indica* variety, IR 54. Calli of the *Japonica* varieties remained compact and smooth while that of the *Javanica* variety became granular in appearance. Histological observation of the resin sections of the calli in all varieties showed darkly stained meristematic cells. The meristematic

cells in *Indica* variety, IR 54, were found in the inner portion of the callus mass while that in the *Japonica* varieties were observed in the peripheral area of the callus mass. The meristematic cells in *Javanica* variety, Rinatte, were scattered all over the callus mass. Scanning electron microscopy revealed distinct differences in the ultrastructures of the callus derived from the different varieties studied. Calli of *Indica* variety had compact cell mass with dome-like structures while callus in the *Japonica* and *Javanica* varieties had compact, globular cell masses.

KEY WORDS

callus cultures, rice varieties, ultrastructures, histology, scanning electron microscopy

INTRODUCTION

The nature and type of callus is one of the most important factors to consider in rice improvement through conventional plant breeding and tissue culture. The embryogenic potential of a variety can be gauged through the type of callus it produced. Callus type is usually evaluated by visual observation. Although visual observation offers some judgment in the selection of good quality callus, it cannot provide information on cell composition and ultrastructures. Histological and scanning electron microscopic observations are the effective tools in determining

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the cell composition and structures of different callus types with potential for regeneration.

Histological studies have been done in rice calli (Nishimura and Maeda 1977; Jones and Rost 1989; Mendoza and Futsuhara 1992). All these studies used histological methods to observe callus induction, somatic embryogenesis and plant regeneration but none focused on the cell composition of observed callus types. The cell composition in a callus mass is important because of its apparent relation to embryogenic potential (Narciso and Hattori 1995).

In this study, the ultrastructure and cell compositions of different callus types derived from selected rice varieties were evaluated visually and by scanning electron microscopy.

MATERIALS AND METHODS

Selected rice varieties belonging to three (3) subspecies namely: *Indica*, *Japonica* and *Javanica* were used in this study. There were five *Indica* varieties, IR 54, Rc 2, 2757, 2764 and Toboshi; two *Japonica* varieties, Tsutsu and Nipponbare and two *Javanica* varieties, Rinatte and Lemonte. The different varieties and their grain characteristics and origin are presented in Table 1.

Dehusked seeds of all varieties were surface sterilized in 70% alcohol for 2 minutes, then in 5% sodium hypochlorite for 3 minutes and finally rinsed three times with sterile distilled water.

The sterilized seeds were inoculated in the following callus induction media:

- 1) Callus induction medium developed by Raval and Chottoo (1993) for *Indica* composed of N6 salts

and vitamins (Chu et al. 1975) + 2.5 mg l⁻¹ 2,4D + 0.015% (w/v) casein hydrolysate + 0.023% (w/v) proline + 0.3% (w/v) sucrose+ 0.8% (w/v) agar herein referred to as IM and;

- 2) Callus induction medium developed for *Japonica* containing N6 salts and vitamins + 2 mg l⁻¹ 2,4 D + 0.003% (w/v) casein hydrolysate + 0.0115% (w/v) proline + 0.35% (w/v) sucrose + 0.025% (w/v) gelrite hereafter referred to as JM.

The pH of the media was adjusted to 5.8 prior to addition of the gelling agent. Forty ml of the medium were dispensed in a 100ml-clear culture bottle, covered with autoclavable plastic and autoclaved at 1.2kg/cm² and 121° C for 20 min. The cultures were placed in a culture room under continuous light (5,000 lx) at 25+/- 2°C.

Visual observation of callus type of each variety in the corresponding callus induction medium was evaluated 2 and 4 weeks after culture. Scanning electron and light microscopic observations were done 4 weeks after culture.

For the scanning electron microscopic (SEM) observation, samples were dipped in liquid nitrogen and viewed under a Hitachi S-2300 scanning microscope with cryo stage. Histological observations under light microscope of the resin sections of the callus samples were prepared according to the method of Mendoza et al 1993. The resin embedded samples were sectioned at 6 µm on a rotary microtome using carbon steel knives and stained with 0.05% toluidine blue for 2 minutes.

The one month old calli of each variety were transferred to two regeneration media:

- 1) Regeneration medium as described by Raval and Chottoo (1993) designated as IRM and;

Table 1. Origin and grain characteristics of selected rice varieties used in the study.

Subspecies/variety	Origin/Source	Grain characteristic
<i>Indica</i>		
IR 54	Philippines	White, slender long
Rc 2	Philippines	White, slender long
2757	Philippines	White, slender long
2764	Philippines	White, slender long
Toboshi	Japan	Red, slender long
<i>Japonica</i>		
Tsutsu	Japan	Red, round short
Nipponbare	Japan	White, round short
<i>Javanica</i>		
Rinatte	Italy	White, roundish long
Lemonte	USA	White, roundish long

- 2) Regeneration medium consisting of $\frac{1}{4}$ Murashige and Skoog (MS) salts and vitamins + $\frac{1}{4}$ N6 salts and vitamins + 0.3% (w/v) sucrose + 2.5 mg l⁻¹ Naphthaleneacetic acid (NAA) designated as JRM.

The regeneration potential was evaluated 4 weeks after culture.

RESULTS AND DISCUSSION

All varieties in general had very good callus formation except for 2764 under IM and Lemonte in IM and JM. The two varieties along with 2757 and Rc2 had poor callus growth which made it difficult to effectively evaluate their callus types. To have a fair comparison, evaluation was done only in varieties with very good callus formation and growth namely IR 54, Toboshi, Nipponbare, Tsutsu and Rinatte. Khaleda and Al-

Forkan (2006) also reported differences in callus growth of Bangladesh varieties belonging to *Indica* subspecies when inoculated in the same medium.

Visual observation of callus morphology

In barley, Bregitzer (1992) found that a callus type was often a characteristic of a genotype. Similar observation was noted in this study. The callus types of each subspecies differed from one another.

Callus type of each subspecies differed from one another under IM. At 2 weeks after culture, calli of *Indica* varieties IR 54 and Toboshi were characterized by the presence of two types of callus masses: one was light yellow, compact, smooth surfaced and the other was yellowish with hair-like projections and tiny green spots. Khaleda and Al-Forkan (2006) also reported embryogenic type of callus in *Indica* rice varieties



Figure 1. Grain (top) and callus morphology (bottom) of IR54 (A) with the green portions of the callus mass, yellowish and compact callus of Nipponbare (B), and yellowish, granular callus of Rinatte (C) under IM 4 weeks after culture.

which was characterized as compact, yellowish and big in size. Calli of *Japonica* varieties, Nipponbare and Tsutsu, and Javanica variety, Rinatte, were yellowish, compact and had smooth surface. However, callus of the *Japonica* varieties was compact and globular, while in that *Javanica* variety, the callus was granular in appearance.

Under JM, callus types of all varieties were almost similar to that in IM, except for IR 54 in which green spots were not evident.

Four weeks after culture, slight changes were observed in the calli of all varieties under both types of induction media. In IM, callus of IR 54 and Toboshi appeared to be water-soaked. In IR 54, greening of more than half of the whole callus mass was

noted while the progression of green spots was not observed in Toboshi (Fig 1A). Calli of Nipponbare and Tsutsu had the same yellowish color but became globular which can be detached easily into small pieces (Fig 1B). On the other hand, calli of Rinatte became granular (Fig 1C). Under JM, no marked morphological change in the calli of all varieties was observed.

Histological observation

A particular callus type has a distinct cell composition and structure. This relationship has been established in crops such as mungbean (Narciso and Hattori 1995), maize (Welter et al. 1995) and leek (Buiteveld et al. 1994). The results of the present study also suggest the same relationship. The characterization of the different callus types showed that the difference in external features correspond to the internal cell composition as shown by

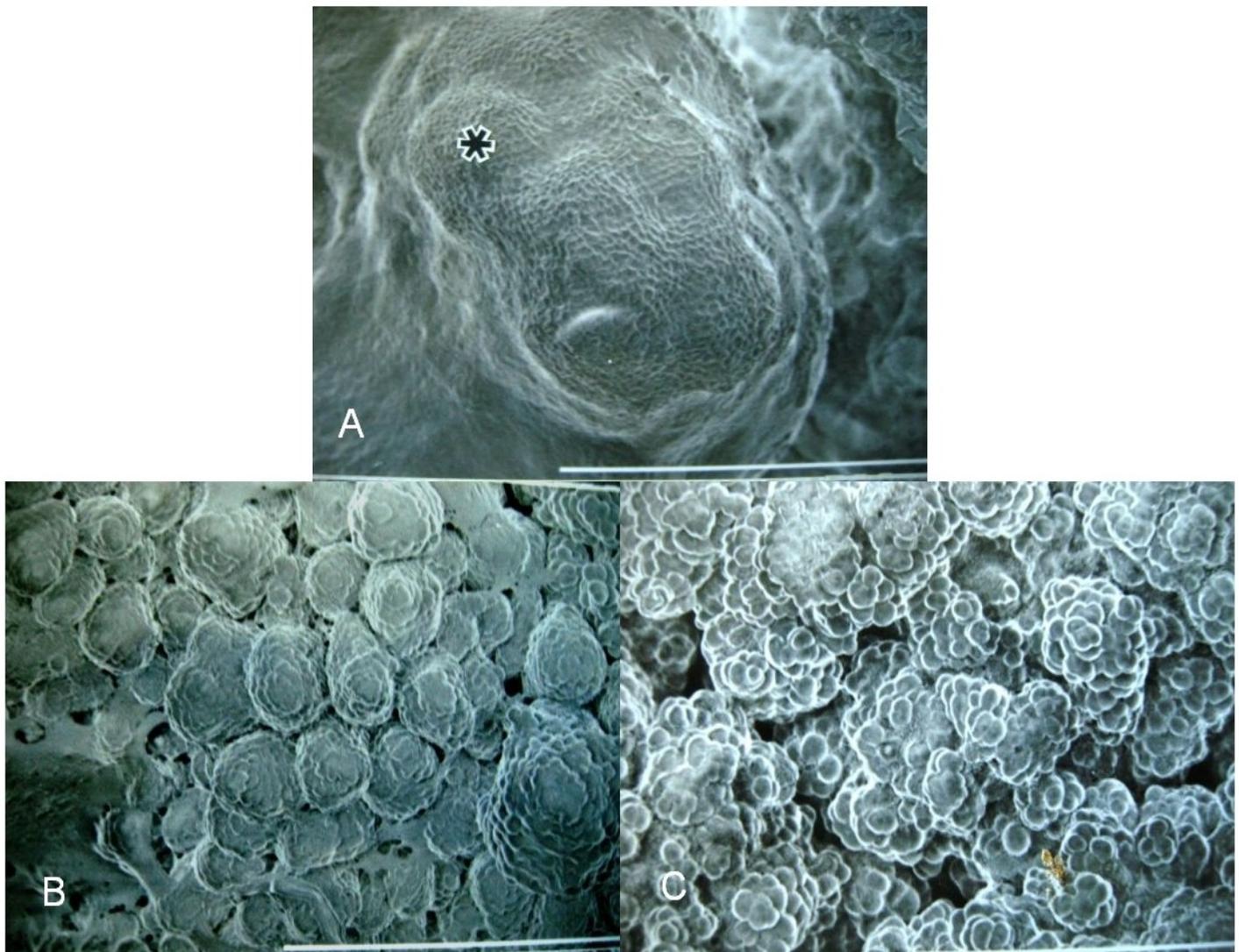


Figure 2. Scanning electron microscopic observation of the callus culture of IR 54 (A) showing the compact cell mass and dome-like structure (*) in IM average length and diameter = 212.50 x 141.70 μm ; Nipponbare (B) with globular, compact cell masses in JM, average length and diameter = 59.40 x 55.20 μm ; and Rinatte (C) with small, round, compactly arranged cells in JM, average length and diameter = 12.50 x 11.40 μm . Bar=200 μm .

the histological and SEM observations.

Meristematic cells were observed in all callus types. These cells appeared as darkly stained and were relatively smaller in size compared to the surrounding cells. Globular patches of meristematic regions were observed in the callus mass of IR 54, Nipponbare and Tsutsu under both induction media. Formation of these regions differed from each variety. In IR 54, the meristematic regions were located in the inner part of the callus

mass (Fig 2A) while in Nipponbare and Tsutsu the regions were found in the peripheral area (Fig 2B). Similar results were noted by Vega et al. (2009) in the embryogenic calli of a local rice variety cultured in a callus induction medium. Epithelial cells were also found proliferating at the outer portion of the callus mass in IR 54. The two regions were surrounded by masses of loose, large, parenchymatous cells. In Rinatte, the meristematic cells were found scattered all over the callus mass (Fig 2C).

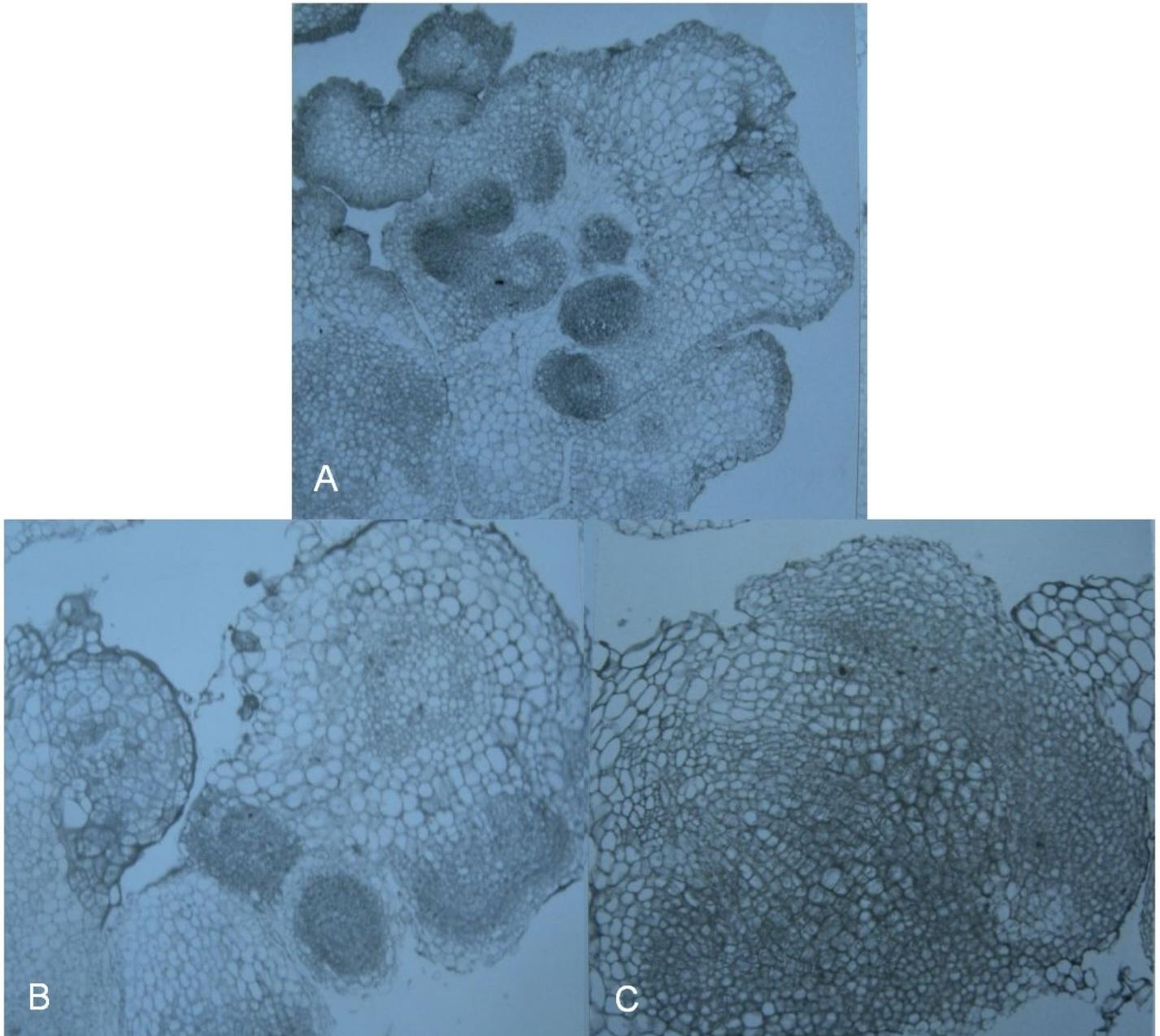


Figure 3. Histological observation of the resin sections of IR 54 (A), Nipponbare (B) and Rinatte (C) showing densely stained meristematic regions .

Scanning electron microscopic observation

SEM observation showed that each callus type had different callus composition. It was also observed that varieties belonging to the same subspecies share similar cell composition and structure. The compact nature of the callus in *Indica* and *Japonica* translate to compact cell mass structure under SEM observations.

IR 54 had a distinctively different callus type in IM and JM. In IM, callus of IR 54 showed slightly differentiated large, highly compact cell mass with dome-like structures (Fig 3A). However in JM, calli of IR 54 showed a large compact cell mass made up of round compactly arranged cells. Similar observation was noted in the calli of Toboshi. The compact cell mass in *Indica* varieties seems to be slightly differentiated showing signs of division. Under SEM, Nakamura and Maeda (1985) observed smooth surface nodules and roughly arranged regions in the callus cultures of *Indica* varieties, Rai-kei and Ai-nan-Tsao after 3 days in the regeneration medium.

Among the *Indica* varieties, IR 54 was the only variety which exhibited green spots in its callus. SEM observation of the callus type in IR 54 showed dome-like structures which are possible sites of shoot initiation. In *Poaceae*, the presence of green spots in cultures has been considered as predictors of potential shoot formation (Nabors et al. 1982).

In *Japonica* varieties, Nipponbare (Fig 3B) and Tsutsu, the calli were made up of compact, globular cell mass of relatively same size. The cell masses were composed of round cells arranged in layers. Nakamura and Maeda (1989) had similar observations.

Calli of Rinatte were composed of small, round, compactly arranged cells (Fig 3C). Sangduen and Klamsomboon (2000) also observed tightly packed cells in the embryogenic callus of the aromatic Thai rice variety.

The results of SEM and histological observations point to the embryogenic potential of all callus types. This can be attributed to the addition of L-proline in the medium. L-proline has been known to promote embryogenesis in maize (Armstrong and Green 1985) and in rice (Chowdhry et al. 1993).

SUMMARY AND CONCLUSION

Visual observation showed that each rice subspecies has a different callus type. Callus type of *Indica* varieties, IR 54 and Toboshi was compact and with hair-like projections. In IR 54, greening of callus mass was also observed. In contrast, the callus type of *Japonica* varieties, Nipponbare and Tsutsu was characterized by its yellowish, globular and callus appearance while that of the *Javanica* variety, Rinatte was yellowish and granular.

The differences in external features of the callus types also

correspond to the differences in ultrastructure as shown by histological and scanning electron microscopic observations. Light microscopic observations showed meristematic clusters in all callus types. In *Indica* varieties, IR 54 and Toboshi, the meristematic regions were located in the inner part of the callus mass while those in *Japonica* varieties, Nipponbare and Tsutsu were found in the peripheral area. In the *Javanica* variety, Rinatte, the meristematic cells were found scattered all over the callus mass.

Scanning electron microscopic observations also revealed differences in cell composition and structure. Large, compact cell masses were observed in the calli of *Indica* and *Japonica* varieties. Callus of Rinatte was made up of small, round, compactly arranged cells. Compact cell masses were not observed in this variety.

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CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Josefina O. Narciso is the principal author who conceptualized and conducted the study with the able guidance of Dr. Kazumi Hattori, the co-author.

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