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NUCLEAR DNA CONTENT IN MERISTEMATIC CELLS OF DORMANT AND GERMINATING EMBRYOS OF RICE SEEDS

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Abstract

Comparisons of G1/G2 distributions in seed embryos of wild, P-10 and cultivar, Taichung-65 rice varieties during germination are conducted with cytophotometry. DNA synthetic period did not exist in resting embryos of both dormant, P-10 and non-dormant, Taichung-65 varieties. After 36 hours imbibition, negligible proportion of cells in P-10 radicle could enter into S period but both proportions of S and G2 in heat-treated P-10 radicle were restored in frequencies similar to that of Taichung-65. On the other hand, almost half of nuclei in P-10 plumule started DNA synthesis but did not terminate the reaction within the 36 hours period. According to results, postulation about mechanism of sprouting resistance in Taiwan wild rice seeds was discussed.

Introduction

Most rice cultivars have lost seed dormancy, and loss of grains often occurs due to seed sprouting in the field before harvest in Taiwan. Understanding of seed germination and dormancy of rice is of great agricultural importance in preservation of grains from sprouting in the field. Studying characteristics of seed dormancy in a wild rice population in Taoyuan, Wu (1978) suggested that the hull and seed-coat were major factors to govern dormancy. He also proposed that the limiting permeability of hulls during imbibition was the primary reason to inhibit the germination in dormant seeds. However, Takahashi (1961) indicated that the dormancy of wild rice was caused not by the seed coat but by their embryo or endosperm. Recently, Kato *et al.* (1977) identified several organic compounds, Koshihikari, which were extracted from seed hulls of poorly germinating cultivar of rice as germination and growth inhibitors. Therefore, complicated physiological events may interact to inhibit germination of dormant rice seed during soaking. Cytophotometric studies of the relative DNA content in the meristematic

nucleus of dry seeds in a number of plants have shown that as the embryo in a seed matures and approaches dormancy, cells in the embryo stop proliferating and are mainly in the presynthetic (G1) and the premitotic (G2) periods of the cell cycle. In *Pinus pinea* and *Lactura sativa* (Brunori and D'Amato, 1976) and *Allium cepa* (Brunori *et al.*, 1970) the nuclei accumulate in G1 whereas in *Vicia faba* (Brunori, 1967), *Triticum durum* (Avanzi *et al.*, 1969), and *Pisum sativum* (Bogdanov *et al.*, 1967) in both G1 and G2. Avanzi (1963) also demonstrated that the G1/G2 ratio of nuclei in the embryonic radicle of dry seeds were variable among varieties of *Triticum durum*. Congar and Carabia (1976) found that approximately equal proportions of G1 and G2 nuclei existed in the root but 5 times as many G1 as G2 nuclei in the shoot of resting maize embryos. After 96 hours soaking in water at 20°C, the cell populations in maize shoot changed so that approximately 33% of cells were in G1, 16.5% in S, and 39% in G2.

In this report, the distribution of G1 and G2 nuclei in the resting embryos of rice seeds of dormant and nondormant varieties were determined. In addition, proportions of G1 and G2 nuclei in the meristems of rice embryos during soaking in water were compared in order to elucidate the mechanism of seed dormancy in rice.

Material and Methods

Plant Materials and Growth Conditions

Seeds of the dormant Taiwan wild rice family designated P-10 (Wu, 1978) and the normal germinating cultivar, Taichung-65, obtained from Dr. Y. T. Kiang, Academia Sinica, Taipei, Taiwan were used in this study. The imbibition of hulled seeds was conducted at room temperature in petri dishes containing cotton saturated with distilled water. P-10 seeds were not able to germinate without breaking dormancy by incubating dry seed for five hours at 60°C before soaking.

Processing Embryo

Three treatments were compared in sequent periods during germination: seed embryos of (1) Taichung-65 (2) P-10 (3) P-10 incubated at 60°C for 5 hours before soaking. Two embryos in each treatment were separated from their endosperms of soaking seeds with a dissecting microscope at 12 hours intervals from initiation of soaking to the end of 36 hours. Dissected embryos were fixed in Carnoy solution for 18 hours. After being dehydrated with N-butanol alcohol and embedded in paraplast, longitudinal sections of embryos in 5μ thick were made. Median sections were mounted, and stained with

Schiff's reagent by the Feulgen reaction (Lillie, 1954).

Measurement of DNA Content in Nuclei

The relative amount of DNA per nucleus was estimated at 560 nm by measuring Feulgen absorption using Reichert Zetopan Spectrophotometer attched to a microscanning stage. Only whole, uncut nuclei which weren't obscured by overlapping nuclei were selected for measurement. G1 and G2 stages of interphase nuclei were determined from relative DNA amounts in telophase (2C) and metaphase (4C) respectively. Measurements of DNA content in individual nuclei of plumule as well as radicle were taken in each section. Thirty nuclei were scored in each meristematic tissue per section except for imbibition periods of 24 hours and 36 hours in plumule of Taichung-65 and 12 hours in the plumule of P-10. Telophase and metaphase cells were made from squash preparations of root tips and then stained with Feulgen reaction. Each twenty DNA values in telophase and metaphase cells were separately taken from ten cells in Taichung-65 and ten cells in P-10 root tip preparations (Fig. 2).

Results

The relative DNA content in interphase nuclei in the embryonic plumule and radicle of three treatments over a 36 hours soaking period were compared. In the resting meristems (zero hour imbibition) there are only a small number of cells within the region of DNA content between 2C and 4C values (Figs. 1 and 3). Therefore, the stage of DNA synthesis (S) should not exist in the resting radicle and plumule. In a resting plumule, 20% nuclei in Taichung-65 and 17% nuclei in P-10 accumulated in G2, where in the resting radicle, 17% nuclei in Taichung-65 and none in P-10 accumulated in G2. Therefore, resting P-10 radicle consisted of least number of G2 cells than that of other resting meristems in the mature embryos. After imbibition, all stages of G1, S, and G2 in interphase would appear in the meristems of germinating embryos (Fig. 1 and 3). Tables 1 and 2 showed the percentages of G1, S, and G2 nuclei in the respective plumule and radicle among 3 treatments during imbibition. Less than 10% of G1 cells in the P-10 radicle of each imbibition period could enter into the DNA synthesis period but more than half of the nuclei in heat-treated P-10 radicle were found in the S period. There is no G2 cell in P-10 radicle during 36 hours imbibition. However, G2 proportions in heat-treated P-10 radicle were restored in a frequency similar to that of Taichung-65. On the other hand, over 36 hours imbibition, almost half of the nuclei in each P-10 plumule were found in S period. Nevertheless, the differences of G2 percentages among three sequent imbibition periods in

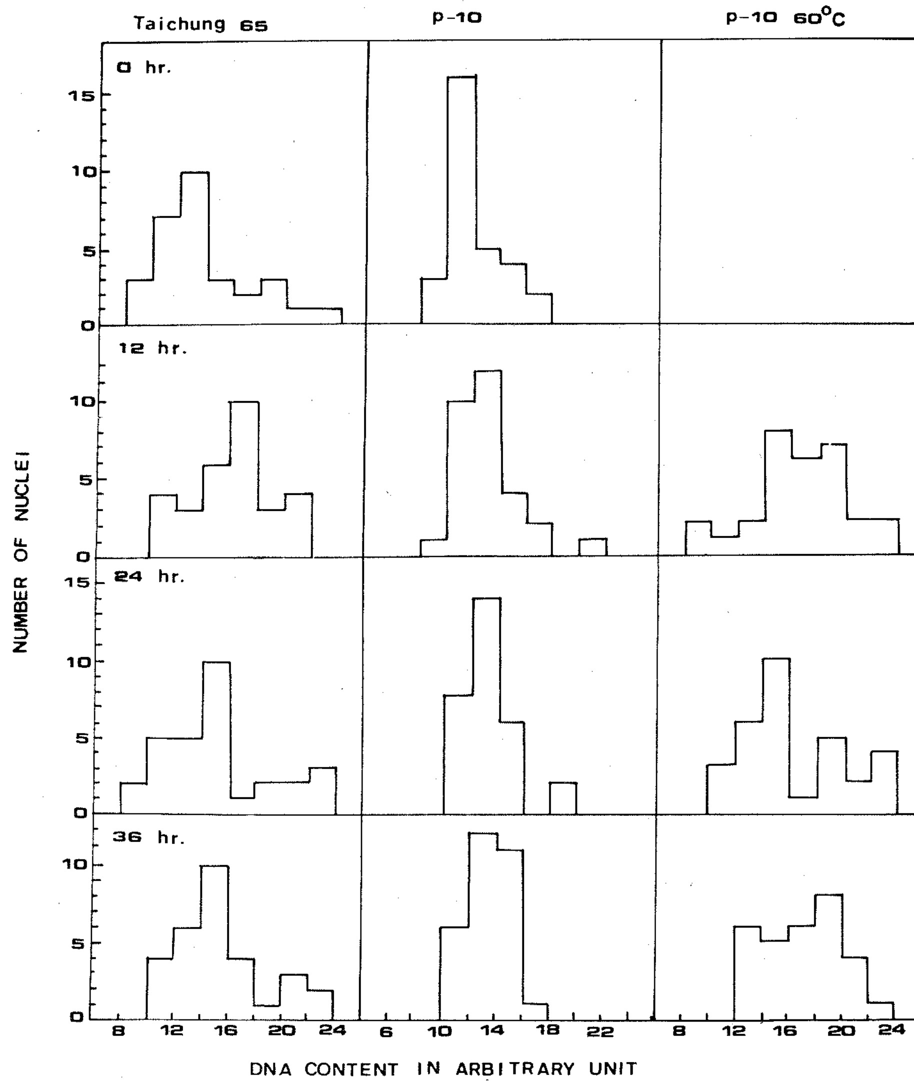

Fig. 1. DNA content of individual nuclei in the plumule of Taichung-65, P-10, and P-10 incubated at 60°C seeds at different imbibition periods.

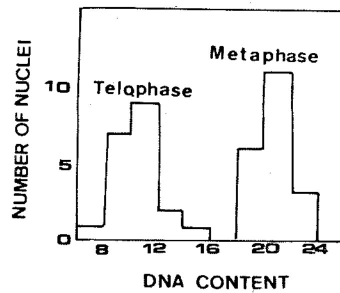

Fig. 2. DNA content of dividing nuclei in the root tips of Taichung-65 and P-10.

Table 1. *Estimation of the percentages of cells in G1, S, and G2 in plumules of Taichung-65, P-10, and P-10 incubated at 60°C during periods of imbibition*

Imbibition	Taichung-65			P-10			P-10 60°C		
	G1	S	G2	G1	S	G2	G1	S	G2
0 hour	70	10	20	73	10	17			
12 hours	23	47	30	29	61	10	13	70	17
24 hours	35	39	26	40	47	13	27	50	23
36 hours	25	50	25	40	53	7	20	60	20

The above estimation was made from data shown in Fig. 1.

Table 2. *Estimation of the percentages of cells in G1, S, and G2 in radicles of Taichung-65, P-10, and P-10 incubated at 60°C during periods of imbibition*

Imbibition	Taichung-65			P-10			P-10 60°C		
	G1	S	G2	G1	S	G2	G1	S	G2
0 hour	67	16	17	84	16	0			
12 hours	23	64	13	90	10	0	17	70	13
24 hours	40	43	17	93	7	0	30	50	20
36 hours	33	50	17	97	3	0	20	63	17

The above estimation was made from data shown in Fig. 3.

dormant P-10 plumule are not significant. Therefore, although a high proportion of cells in P-10 plumule entered into S period during imbibition, they were not able to accomplish DNA synthetic activity within a 36 hours period.

The mitotic index represents the proportion of nuclei in all phases of mitosis. The mitotic figures (Fig. 4) showed that there was no mitotic activity in resting meristems of both Taichung-65 and P-10 except of a small fraction of mitotic index ($I = 1.5$) in the plumule of P-10. During soaking, hardly any dividing cells were found in both plumule and radicle of dormant P-10 seeds. In contrast, cells in both meristems of heat-treated p-10 were proliferating in a rate comparable to that of Taichung-65.

Discussions

A small percentage of nuclei containing 2C-4C intermediate values of DNA were observed in the resting embryo of P-10. Most of them are probably actually either 2C or 4C nuclei. A certain degree of error is inherent to the cytophotometry as well as to differential and imcomplete staining of cells.

In contrast to previously published data for resting radicles of some other

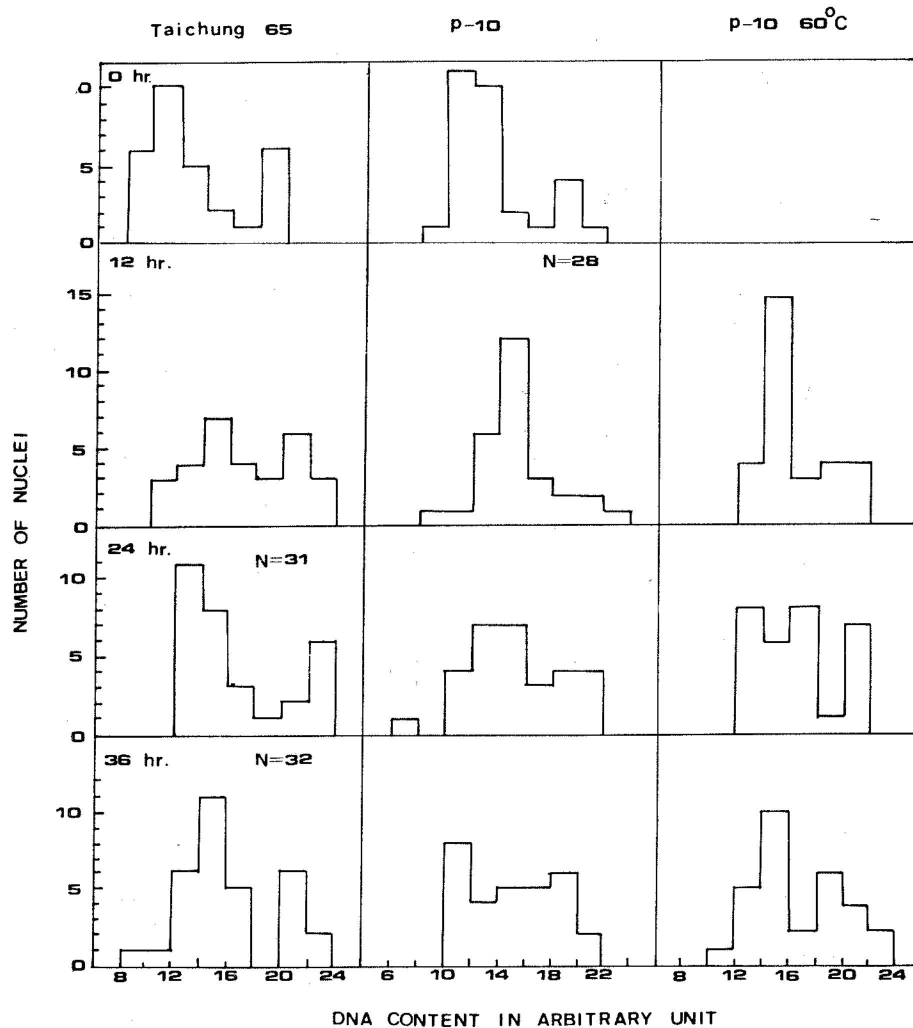

Fig. 3. DNA content of individual nuclei in the radicle of Taichung-65, P-10, and P-10 incubated at 60°C seeds at different imbibition periods.

species such as *Triticum durum* (Avanzi *et al.*, 1969), *Pisum sativum* (Bogdanov *et al.*, 1967), and *Zea mays* (Conger and Carabia, 1976) showed a mixed cell population of G1 and G2, the present data in dormant radicle of P-10 showed predominantly 2C cells. Similar observations have been reported on the resting radicles of *Lactura sativa* and *Pinus pinea* (Brunori and D'Amato, 1967) and *Festuca arundinacea* and *Dactylis glomerata* (Congar and Carabia, 1978).

Avanzi *et al.* (1969) found that different meristems of dry embryos of *Triticum durum* had different ratios of G1/G2 nuclei. They suggested that G2 nuclei existed in the radicles because the water content of the seed dropped below a critical point before these G2 nuclei underwent mitosis and became

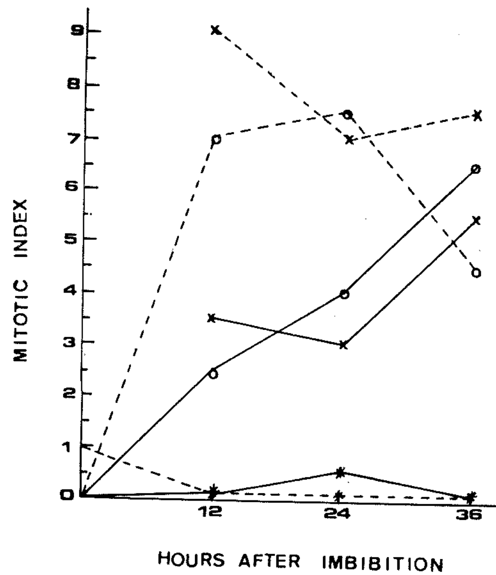

Fig. 4. Mitotic index in meristems of Taichung-65 and heat treated seeds. (o: Taichung-65; * : P-10; × : P-10 60°C; —: Plumule; ---: Radicle)

G1 nuclei. Whereas the plumule completed their development before the critical point of water content was reached thus allowing depletion of the G2 nuclei in the plumule by mitosis. Brunori (1967) indicated that in ripening *Vicia* seeds DNA synthesis was terminated before the mitosis ceased. Thus, they suggested that DNA synthesis was more sensitive to dehydration of the seed than was mitosis. From the above evidence, Jones (1977) suggested that the duration between inhibition of DNA synthesis and inhibition of mitosis in a maturing embryo determined the phase of depletion of G2 nuclei and that the rate of loss of water from the embryo induced the depletion of G2. Therefore, the frequency of G2 cells in mature embryo is inversely proportioned to the rate of water loss during embryo maturation. The evidence presented in this paper suggested that the slowest rate of water loss occurred in the cells of P-10 radicles during seed maturation. A high water affinity of cells may be responsible for a slow rate of water loss. In this case, a P-10 radicle should have a highest water affinity than any other meristem in the experiment. The results also show that even cells in dormant plumule of P-10 are able to absorb water by the process of imbibition to initiate DNA synthesis during imbibition. Thus, the silent radicle of P-10 without metabolic activity (DNA synthesis) during imbibition does not appear to be lack of hydration in cellular components. Therefore, the present results disagree with the hypothesis proposed by Wu (1978) who stated that the dormant factor in P-10

seeds was due to the inhibition of permeability by seed hulls during soaking. Since he did not find any germination inhibitors in seed hulls, a limiting mechanism for seed germination might exist in the embryo or endosperm (Takahashi, 1961). Rost and Van't Hof (1973) studied cell proliferation of an excised root tip of *Helianthus annuus* in sucrose-deficient medium and found that most cells in the meristem accumulated in G1 but a small proportion of cells continuted through DNA synthesis. Since continuous cell proliferation in the meristem required a high level of metabolism and if they starved in carbohydrates, a stationary condition was produced with cells arrested in G1 or G2 (Van't Hof and Kovaces, 1972). They called these continuous DNA synthesizing cells of sunflower in a sucrose deficient medium as leaky cells. A small number of cells in the dormant radicle of P-10 which was capable of synthesizing DNA during imbibition may have a similar leaky property. Although a substantial proportion of cells in dormant plumule of P-10 initiate DNA synthesis during imbibition, their growth is still limited since they are unable to complete their S period and subsequent cell cycle.

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休眠性及發芽性水稻種子中再生細胞核DNA含量

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利用 Cytophotometry 比較野生稻種子及栽培品種種子在發芽過程中胚細胞 G1/G2 的分佈。休眠性稻種 P-10，和非休眠性稻種臺中65之胚細胞在 resting stage 均不具有 DNA 之合成期。P-10 radicle 經過36小時之浸泡後，可忽略比例之胚細胞能進入 DNA 合成之 S 期，但是 P-10 經過熱處理後則其 S 和 G2 期可以恢復到與臺中65稻種之S 和 G2 相似之比例，另一方面，在36小時之內幾乎半數的 P-10 plumule 細胞核可開始合成 DNA 而不會終止此種合成的反應。由於本文的結果臺灣野生稻種子休眠性的機制予以推測。