Genetic Variations in Cell and Tissue Forms in Relation to Plant Growth

I. Relationship between Growth Rates of the *Brassica* Species and Cell Sizes of the Shoot Apex and Palisade Parenchyma

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Synopsis. Species with higher relative growth rates exhibited higher relative surface exposures at both plant and cellular levels. In meristematic cells enriched with cytoplasm, a decrease in cell-size brings about an increase in the ratio of cell surface area to cytoplasmic volume. In vacuolated cells, e.g., cortex and palisade parenchyma, an increase in cell-size with vacuolation brings about an increase in the ratio of cell surface area to cytoplasmic content. Rapid growth was associated with higher leaf area ratio, smaller cell-size in meristematic cells, and larger cell-size in vacuolated cells.

Introduction

Close associations exist between the growth of plants and the forms of their organs, tissues and cells. SCHWANITZ (1953) emphasized the importance of cell size in the phylogeny and ontogeny of plants, MAKSYMOWYCH (1969) dealt with cell number in relation to plant growth, and relationships between cell size and photosynthetic activity were shown by EL-SHARKAWY, *et al.* (1965), WILSON, *et al.* (1967) and SASAHARA, *et al.* (1968).

TSUNODA (1964) stated that the productive systems of plants had been clarified, to some extent, at the population-plant-organ level, however, investigations at the tissue-cell-organelle level had to be advanced to reach a more complete picture of the plant productive system. In this paper, variations among *Brassica* species in cell size in the shoot apex and in the palisade parenchyma are described in relation to plant growth, total cell number per plant and average cell weight.

Materials and Methods

Materials used are listed in Table 1. These strains belong to genetic stocks of *Brassicaceae* maintained at the Faculty of Agriculture, Tohoku University Sendai. Strains with one asterisk were used in 1964 and strains with two asterisks were used in 1965.

Experiment 1964

A randomized block design with four repli-

cations was adopted. One block was formed by a concrete frame (120 cm × 300 cm, 10 cm in depth) filled with sieved loam soil mixed with ammonium sulfate, super phosphate and potassium chloride containing 14.4 g nitrogen, 7.6 g P₂O₅ and 10.4 g K₂O, respectively.

Fifty seeds of each strain were sown in a row in each block on May 13. Seven days later, a total of 20 plants, 5 from each block, were harvested with roots, and bulked as the first sampling. Then, 12 plants, 3 from each block, were harvested at 7 day intervals as the second, third and the fourth samplings. Harvested plants were washed in water, and the leaves were excised. Leaf area was determined by copying leaves on Fuji-copica paper with a certain weight-to-area ratio.

Relative growth rate, leaf area ratio and the net assimilation rate were computed for each interval between two samplings based on the following formulae:

$$\text{RGR} = (\text{Ln } W_2 - \text{Ln } W_1) / (t_2 - t_1)$$

$$\text{LAR} = 1/2(A_2/W_2 - A_1/W_1)$$

$$\text{NAR} = \text{RGR} / \text{LAR}$$

where W_2 , W_1 , and A_2 , A_1 show the total dry weight and leaf area at times t_2 and t_1 , respectively (BLACKMAN, *et. al.*, 1951 and RADFORD, 1967).

To determine cell size in the palisade parenchyma, fully expanded first leaves were fixed in FAA solution at 4 weeks after sowing. Leaf-disks (1 cm. sq.) were cut from the middle portion of the half-leaf (including as few veins as possible), then dehydrated through an

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Table 1. Materials with their genomes

Species	Strain		Genome
<i>Brassica campestris</i> L.	C-101*, **	Hatana-4 turnip rape	AA
	C-213*	Kyoto-3 chinese cabbage	"
	C-333*	Yukina turnip green	"
	C-430*	Tokinashi-Shirokokabu turnip	"
<i>B. nigra</i> Koch.	Ni-101**	California-Brown	BB
<i>B. oleracea</i> L.	O-3*	Kairyo-Nambu cabbage	CC
	O-8*	Yoshin tropical cabbage	"
	O-9*	New Kale	"
	O-144*	Kohlrabi	"
	O-25**	Cauliflower	"
<i>B. juncea</i> Coss.	J-103**	Murasaki-Takana	AABB
<i>B. napus</i> L.	N-101*, **	Yotsukaichi-Kurodane rape	AACC
	N-261*	Svalöf-Victory swede	"
	N-343**	Early-Giant fodder rape	"
	N-404*	(from Hokkaido Univ.)	"
<i>B. carinata</i> A. Broun.	Ca-101**	Harron	BBCC
<i>4x-campestris</i>	4xC-101*	Autotetraploid of C-101	AAAA
	4xC-213*	" C-213	"
<i>4x-oleracea</i>	O-136*	Swedish marrow stem kale	CCCC
Artificial <i>napus</i>	CO-1*	(from Konosu Expt. Stn.)	AACC
	CO-2*	(from Utsunomiya Univ.)	"
	CO-3*	(")	"
	CO-44*	(from Svalöf)	"
	CO-46*	(from Kyushu Univ.)	"
	CO-50*	Cabusai	"
	NapC-1*	Winter variety (from Svalöf)	AAAACC
<i>Napocampestris</i>	NapC-2*	Summer variety (")	"

Notice : Strains marked with one asterisk were used in 1964, those with two asterisks in 1965.

alcohol series, embedded in paraffin and stained by the following *chloroform-gentian violet* method ;

1) Treat in chloroform solution containing 5% gentian violet for 45 min. In this procedure, paraffin was removed and specimens were stained.

2) Bleach in chloroform solution.

3) Rinse in an equal volume mixture of chloroform and xylem for 10 min.

4) Rinse in xylem for 10 min. (three times).

5) Mount in Canada balsam.

Average cell sizes were estimated on photographs of 20 cells randomly selected using the method reported for leaf area determination.

To determine cell size in the shoot apex, plants were sampled at the cotyledon stage, and transverse sections were cut 5 μ thick. Other procedures were the same as in the palisade parenchyma.

Experiment 1965

Plants for the estimation of total cell number were sown on Sept. 13. Methods of nursery preparation and management were the same as in the 1964 experiment. Cell number was determined according to the method of MAKSYMOWYCH (1963). Separated cells were suspended in 50% glycerin solution, then using a blood corpuscle counting chamber (Erma, Tokyo, 0.2 mm in depth), ten counts were made per material.

Results

1. Growth analysis - Leaf area ratio and relative growth rate

Changes in total dry weight and total leaf area at successive sampling times are shown in Figs. 1 and 2, respectively, for *campestris*, *4x campestris*, *oleracea*, *4x oleracea*, *napus* and artificial *napus*, as mean values of strains of each group. Of the three natural species, the relative growth rate was highest in *campestris*, lowest in *oleracea*, and intermediate

in *napus*. Autotetraploids showed a lower relative growth rate as compared with the two natural diploids of *campestris* and *oleracea*. However, artificial *napus* (amphidiploids of *campestris* and *oleracea*) exhibited a slightly higher relative growth rate than natural *napus*.

A clear positive correlation existed between leaf area ratio (LAR) and relative growth rate (RGR) as shown in Fig. 3. In this experiment, plants were sampled at the rosette stage, therefore most of the total dry weight was held by leaves. This means that leaf area ratio (leaf area : dry weight ratio) was determined mainly by specific leaf area (leaf area : leaf dry weight ratio). Thus, *Brassica campestris* which developed thin leaves with a high specific leaf area was high in its relative growth rate; *oleracea*, which developed thick leaves with a low specific leaf area, was low in its relative growth rate; and

strains such as *napus*, artificial *napus*, and *napocampestris* which were intermediate in leaf thickness (specific leaf area) were also intermediate in their relative growth

Fig. 1. Changes in dry weight
○ *campestris*, ● *4x-campestris*, □ *oleracea*,
■ *4x-oleracea*, △ *napus*, ▲ artificial *napus*

Fig. 2. Changes in leaf area

○ *campestris*, ● *4x-campestris*, □ *oleracea*,
■ *4x-oleracea*, △ *napus*, ▲ artificial *napus*

Fig. 3. Relationship between leaf area ratio and relative growth rate during 1-4 weeks after planting in 1964

○ *campestris*, ● *4x-campestris*, □ *oleracea*,
■ *4x-oleracea*, △ *napus*, ▲ artificial *napus*,
▲ *napocampestris*

rates. Autotetraploids of *campestris* and *oleracea* with "thicker leaves" were lower in their relative growth rates as compared with two diploids of *campestris* and *oleracea*, respectively.

A similar positive correlation between leaf area ratio and relative growth rate was also observed among materials tested in 1965, as shown in Fig. 4. Two amphidiploids, *napus* and *carinata*, were intermediate in leaf area ratios and relative growth rates as compared with their parental species; *napus* being the amphidiploid between *campestris* and *oleracea*, and *carinata* the amphidiploid between *nigra* and *oleracea*. Another amphidiploid, *juncea*, was as high as *campestris*, one of the parental species, in leaf area ratio and in relative growth rate (Fig. 4).

Fig. 4. Relationship between leaf area ratio and relative growth rate during 1-2 weeks after planting in 1965

○ *campestris*, × *nigra*, □ *oleracea*,
⊗ *juncea*, △ *napus*, ⊠ *carinata*

2. Cell size in shoot apex and relative growth rate

Relative cell size in the shoot apex increased gradually in a top to basal direction. The increasing rate was highest in *campestris*, intermediate in *napus*, and lowest in *oleracea* among the natural species, as shown in Fig. 5.

The size of cells located within a 0.4 mm square from the top surface of the shoot apex, including tunica-corporus layer cells, was markedly small in *campestris* and large in *oleracea* and *napus*. This difference in cell size became smaller closer to the basal portion. The size of cells located more than 1.6 mm from the top surface was rather large in *campestris*, intermediate in *napus*, and small in *oleracea*. The turning point of this reversion in cell size was about 1.5 mm from the top surface of the shoot apex, where cells initiated

vacuolation and differentiation becoming cortex cells etc.

Thus, the size of dividing cells in meristematic tissue of the shoot apex, including tunica-corporus layers, is markedly small in *campestris* while showing a higher relative growth rate as compared with *oleracea* and *napus*. The size of vacuolated and differentiated cells at the basal part of the shoot apex, including cortex cells, is rather large in *campestris* exhibiting a high growth rate.

Relationship between cell size in the tunica-corporus area and relative growth rate is shown in Fig. 6.

Fig. 7 shows the relationship between cell sizes in the tunica-corporus area and leaf number 4 weeks after planting.

Fig. 5. Variations in cell size in the shoot apex
○ *campestris*, ● *4x-campestris*, □ *oleracea*,
△ *napus*, ▲ artificial *napus*

*Relative cell size = 1/cell number in 0.4 mm square

Fig. 6. Relationship between relative cell size in the tunica-corporus area of the shoot apex and relative growth rate

○ *campestris*, ● *4x-campestris*, □ *oleracea*,
△ *napus*, ▲ artificial *napus*

* See Fig. 5.

Fig. 7. Relationship between relative cell size in the tunica-corpora area of the shoot apex and leaf number per plant. Leaf number was counted 4 weeks after planting, omitting leaves less than 10 mm long.

○ *campestris*, ● *4x-campestris*, □ *oleracea*,
△ *napus*, ▲ artificial *napus*, ▲ *napocampestris*
* See Fig. 5.

3. Cell size in the palisade parenchyma, relative growth rate and net assimilation rate

Average cell sizes in the palisade parenchyma for six groups are shown in Fig. 8 on emerged leaves in plants harvested 4 weeks after sowing. Cell size changed with the leaf order, being highest in the first expanded leaves and smallest in the top expanding leaves. However, cell size in the palisade parenchyma was small in *oleracea* as compared with *napus* and *campestris*.

In contrast to meristematic cells of the shoot apex, the size of photosynthetic cells was rather large in *campestris*, showing a higher relative growth rate, as compared with *oleracea*.

The relationship between cell size, in the palisade parenchyma, of the first expanded leaves and net assimilation rate 3-4 weeks after sowing, is shown in Fig. 9. As shown, the relationship between cell size and net assimilation rate was not clear. We can not say that a negative correlation between the cell size of palisade parenchyma and the net assimilation rate existed.

4. Cell number per plant, mean dry weight per cell, cell size and plant growth

For materials tested in 1965, both cell size and total cell number were observed in relation to plant growth. Changes in total cell number at successive samplings are shown in Fig. 10.

Fig. 11 shows a close correlation between total cell number per plant and total leaf area.

The relationship between total cell number

Fig. 8. Variations in cell size in the palisade parenchyma

○ *campestris*, ● *4x-campestris*, □ *oleracea*,
■ *4x-oleracea*, △ *napus*, ▲ artificial *napus*

Fig. 9. Relationship between cell size in the palisade parenchyma and net assimilation rate. Cell size was observed in the first expanded leaves 4 weeks after planting. Net assimilation rate was calculated for 3-4 weeks after planting.

○ *campestris*, ● *4x-campestris*, □ *oleracea*,
△ *napu*, ▲ artificial *napus*, ▲ *napocampestris*

and total plant dry weight is presented in Fig. 12 showing a quadratic regression. Changes in mean dry weight per cell are shown in Fig. 13.

Based on results shown in Figs. 10 and 13, three groups are designable. 1) *campestris* and *juncea* (rapid growth), 2) *carinata* and *napus* (intermediate) and 3) *oleracea* and *nigra* (slow). The first week after sowing the increase in cell number was not so marked for all species,

however, during this period *campestris* and *juncea* markedly increased in mean dry weight per cell as compared with *oleracea* and *nigra*. The second week after sowing, *campestris* and *juncea* increased in cell number per plant with a very rapid decrease in the mean dry weight per cell. In *oleracea* and *nigra* the increase in cell number was not so rapid and the mean dry weight per cell was still increasing during this period.

At the seed stage, the mean dry weight per cell was low in diploid species (*campestris*, *nigra* and *oleracea*) and high in tetraploid

Fig. 10. Variations in total cell number
○ *campestris*, × *nigra*, □ *oleracea*,
⊗ *juncea*, △ *napus*, ⊠ *carinata*

Fig. 11. Relationship between total cell number per plant and total leaf area
○ *campestris*, × *nigra*, □ *oleracea*,
⊗ *juncea*, △ *napus*, ⊠ *carinata*
I one week, II two weeks after planting

Fig. 12. Relationship between total cell number per plant and total plant dry weight.
□ *oleracea*, ○ *campestris*, × *nigra*,
⊗ *juncea*, △ *napus*, ⊠ *carinata*
0 seed, I one week, II two weeks after planting

Fig. 13. Variations in dry weight per cell
○ *campestris*, × *nigra*, □ *oleracea*,
⊗ *juncea*, △ *napus*, ⊠ *carinata*

Fig. 14. Changes in cell size
○ *campestris*, × *nigra*, □ *oleracea*,
⊗ *juncea*, ▲ *napus*, ⊠ *carinata*

species (*napus*, *juncea* and *carinata*). But 14 days after sowing it was low in *campestris*, *juncea* and *carinata*, and high in *oleracea*, *nigra* and *napus* (Fig. 13).

Changes in cell size are shown in Fig. 14 for cotyledons and first leaves of the same materials. As mentioned, the mean dry weight per cell was higher in *oleracea* and *nigra* than in *campestris* and *juncea* 14 days after sowing. On the other hand, as shown in Fig. 14, cell size in leaves tended to be low in *oleracea* and *nigra* as compared with *campestris* and *juncea*. Fourteen days after sowing most of cells in plants were those of leaves, including cotyledons. Thus, the cells of *oleracea* and *nigra* tended to be small in size containing much dry matter, whereas cells of *campestris* and *juncea* were large in size containing less dry matter 14 days after sowing.

Discussion

The significance of internal and external surface exposure of plants, organs and cells has been discussed by several workers in relation to physiological activities. Surfaces of plants, organs and cells are the places where the input and output of molecules and light quanta essential to metabolism take place. Thus, an increase in surface exposure tends to increase physiological activities. HABERLANDT (1965) called this trend in the cell level "the principle of maximum exposure".

The leaf area ratio, i.e., the ratio of leaf area to total plant dry weight, has been used as an index of relative surface exposure for whole plants. BRIGGS and WEST (1920) and others have pointed out that the higher the leaf area ratio, the higher the relative growth rate; provided light and water are supplied adequately to all leaves (e.g. TSUNODA, 1959). In this experiment also, high leaf area ratio was associated with a high relative growth rate (Figs. 3 and 4).

Concerning relative surface exposure at the cell level, the importance of cell size was shown by SCHWANITZ (1950); a decrease in cell size is associated with an increase in the surface to volume ratio which may bring about an increase in physiological activities. EL-SHARKAWY and HESKETH (1965) and WILSON and COOPER (1967) found that species and

strains showing higher photosynthetic activities tended to have smaller sizes of photosynthetic cells.

In this experiment, species showing higher relative growth rates exhibited smaller sizes of meristematic cells in the tunica-cortex area of the shoot apex (Fig. 6). However, a higher net assimilation rate was not necessarily associated with smaller cell size in leaves (Fig. 9). Furthermore, vacuolated cortex cells in the stem are somewhat larger in species with higher relative growth rates.

We conclude that species with higher relative growth rates exhibited higher relative surface exposures at both plant and cellular levels. Rapid plant growth was associated with higher leaf area ratio, smaller sized meristematic cells in the shoot apex, and larger sized vacuolated cells in the cortex and palisade parenchyma. Small cell size in meristematic cells may favor cell division resulting in a rapid increase in total cell number and leaf number (Fig. 7). Increase in cell size with vacuolation may not only increase the surface exposure of the cell but also tends to increase the relative surface exposure at the plant level resulting in; rapid plant growth, increase in cell division, leaf number, etc. Thus, the causal relationships of cell size to the growth characteristics do not appear to be simple and need to be investigated further. We can only say now, that as far as the materials observed in this experiment are concerned, higher relative surface exposures at both plant and cellular levels were associated with rapid plant growth.

Summary

Species showing higher relative growth rates exhibited higher relative surface exposures at both plant and cellular levels. Rapid plant growth was associated with the higher leaf area to plant dry weight ratio, smaller sized meristematic cells in the shoot apex, and larger sized vacuolated cells in the stem cortex and leaf palisade parenchyma.

In cells enriched with cytoplasm, i.e., the meristematic cells of the tunica-cortex area of the shoot apex, small cell size is associated with an increase in the cell surface to cell volume ratio and, simultaneously, the cell surface to cytoplasmic volume ratio. In

vacuolated cells, such as the stem cortex and leaf palisade parenchyma, an increase in cell size may cause an increase in the cell surface to cytoplasmic content ratio which favors relative surface exposures at both plant and cellular levels.

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細胞・組織形態の遺伝変異と植物生長

I. 生長点細胞・柵状細胞の大きさと生長率

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アブラナ属の3種 18 系統, 同質倍数体3系統, 2基6倍体2系統を用い, 生長点細胞・柵状組織細胞の大きさと生長率の関係を検討した。また, アブラナ属の6種6系統を用いて, 生育にともなう個体全細胞数の変化および細胞当り乾物重の変化と生長率の関係を検討した。

1) 高い生長率を示す系統は, 生長点の分裂組織の細胞が小さく, 他方細胞体積のほとんどが液胞によつて占められている cortex 細胞や葉肉柵状組織細胞は大きい傾向がみられた。

2) 細胞内が細胞質で満たされ, 細胞質が比較的均一に分布している細胞 (例えば生長点の tunica-corpus 域の細胞など) では, 細胞の大きさが小さければ小さい程, 細胞体積に対する細胞表面積の比が大きく (細胞質体積に対する細胞表面積の比も大きく) なり, このことが細胞の生理活性を高める結果, 生長率が高くなると推定さ

れる。

3) 一方, 液胞をもつ細胞 (例えば, cortex 細胞, 葉肉柵状組織細胞など) では, 細胞の生理活性にとつて重要な位置を占めている葉緑体, ミトコンドリア, 小胞体, 核などを含有する細胞質部分は細胞壁に薄層となつて展開している。このような細胞では, 細胞の大きさが大きくなればなる程, 細胞質含有物質質量に対する細胞表面積の比が大きくなる (なわち, 相対的表面発達度が大きくなる) なり, 細胞当りの生理活性は大きい細胞において高くなるであろう。

4) 高い生長率を示す系統は, 個体・細胞レベルにおいて, 相対的表面発達度 (葉面積 : 個体乾物重比 = Leaf area ratio および細胞表面積 : 細胞含有物質質量比) が高い傾向がみられた。