

ESTIMATION OF DRY MATTER AND LIGNIN DISTRIBUTIONS IN MAIZE INTERNODES BY MICRO-SURGICAL TISSUE SEPARATION

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ABSTRACT

The second, 5th and 8th internodes, counting from the lowest internode, taken from the heading, milk-ripe and full-ripe maize (*Zea mays L.*), were dissected into fractions of epidermis (EPI), sclerenchyma (SCL), vascular bundle (VB) and pith parenchyma (PITH).

The proportion of dry weight of VB to the total dry weight were smallest (8-13%), followed by EPI (15-22%) and SCL (15-30%) and PITH (43-58%). These values differed entirely from the proportions of area for the fractions on the cross-cut section.

The lignin concentration, in the most case, was in the order of; EPI > SCL > VB > PITH. From the bottom toward the top of stem, overall, lignin concentration increased in PITH but it decreased in EPI and SCL, and in VB was relatively consistent.

The proportion of lignin content in VB to the total lignin content was smallest (9-14%), and varied from 24 to 35% for EPI, from 18 to 48% for SCL and from 12 to 44% for PITH. The higher the internode the smaller the proportion of lignin content in SCL, consequently, the greater in PITH.

KEYWORDS

Internode, Lignin distribution, Maize, Micro-surgery, Plant tissue

INTRODUCTION

Histochemical studies show that forages are composed of various tissues which are varying in structural and digestible characteristics (Kawamura et al., 1973). However, it is difficult to make accurate quantitative analyses in these tissues through a microscope.

Recently, the physical separation of tissues by hand was made satisfactorily for some large-stemmed plants (Hartley et al., 1992; Wilson et al., 1994). A similar technique was adopted in this paper as well as in our previous paper (Kawamura and Nijjima 1996) to estimate quantitatively the dry matter and lignin distributions in maize stems.

MATERIALS AND METHODS

The seeds of maize (cv. Snowdent G4624) were sown in the paper pots on June 1, 1994 and then the vigorous seedlings were individually transplanted into a pot of 1/2000a size and grown outdoors. Four plants were collected on August 2 (heading stage), on August 23 (milk-ripe stage) and on September 13 (full-ripe stage).

Two cross-cut stem sections (c. 30-50mm long) were taken from each central part of the second, 5th and 8th internodes, counting from the lowest internode above the ground.

One of the two sections was dried at 50 to determine the loss of dry matter during dissection.

The other section was dissected into the four fractions, that is; the epidermis fraction (EPI) contained epidermis and the sclerenchyma adhered to the epidermis, the sclerenchyma fraction (SCL) contained

the peripheral sclerenchyma layer (including the ensheathed small vascular bundles), and the vascular bundle fraction (VB) and the pith fraction (PITH) consisted entirely of the large vascular bundles embedded in the pith parenchyma and the pith parenchyma, respectively (Kawamura and Nijjima 1996). Each fraction was dried and weighed, and then subjected to lignin determination.

Additionally, the cross-cut section (c. 200µm thick) from each internode was stained with toluidine blue (Kawamura et al., 1973) for the measurement of areas occupied by the tissue fractions, using an image analysis system (COSMOZONE, Nikon, Japan).

RESULTS AND DISCUSSION

The maize did not increase the height and the length of experimental internodes during experiment. For all internodes, dry matter was almost completely (93-105%) recovered from four fractions as previously reported (Kawamura and Nijjima 1996).

The proportions of dry weight of the fractions to the total dry weight are shown in Table 1. The values for VB were smallest and varied from 8 to 13%, followed by EPI (15 to 22%) and SCL (15 to 30%), and the values for PITH were largest and varied from 43 to 58%. As a result, about 33 to 47% of the internode dry matter was in the epidermis and sclerenchyma or the "rind" and the remaining was in the pith parenchyma and large vascular bundles or the "stele". With maturity, the proportion of PITH increased and of the other fractions decreased in the second and 5th internodes. In addition, the higher the internode the smaller the proportion of the rind in the plants at heading and full-ripe stages.

As shown in Table 1, the proportions of area for the fractions on the cross-cut section differed very markedly from the proportions of the dry weight.

Lignin concentration in each fraction was in the order of; EPI > SCL > VB > PITH for the all experimental internodes except the 8th internode at full-ripe stage. Although lignin concentration did not change largely with maturity in each fraction, from the bottom toward the top of the stem, overall the concentration increased in PITH but it decreased in EPI and SCL, and in VB was relatively consistent. This result was very similar to the previous observation in sorghum (Kawamura and Nijjima 1996).

The proportion of lignin content in the fractions to the total lignin content for each internode at the three stages is shown in Fig. 1. The values for VB were smallest and varied from 9 to 14%, and for the other fractions were relatively high and varied from 24 to 35% for EPI, from 18 to 48% for SCL and from 12 to 44% for PITH. As a result, 42 to 79% of the internode lignin was in the rind and 21 to 58% was in the stele. The proportion of lignin content in each fraction did not change largely with maturity. At each stage, the higher the internode the smaller the proportion of lignin content in SCL, consequently, the greater in PITH. Therefore, the distribution of lignin to the rind decreased (79 to 54% at heading, 77 to 54% at milk-ripe and 67 to 42% at full-ripe stages) and to the stele increased as the internodes went up toward the top of the stem.

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Table 1
Proportions of dry weight of the tissue fractions to the total dry weight of the internode.

<u>Internode</u>	<u>Fraction</u>	<u>Heading</u>	<u>Milk-ripe</u>	<u>Full-ripe</u>
8th	EPI	18.2 (6.6)	21.9 (7.9)	17.3 (3.8)
	SCL	18.1 (13.1)	21.8 (15.3)	15.3 (11.3)
	VB	12.6 (4.6)	9.5 (4.6)	10.9 (6.1)
	PITH	51.1 (75.5)	46.8 (72.2)	56.6 (78.8)
5th	EPI	20.5 (6.2)	16.8 (5.4)	16.5 (4.1)
	SCL	22.2 (10.7)	17.7 (11.5)	16.7 (8.7)
	VB	9.6 (3.3)	9.3 (4.6)	9.2 (5.0)
	PITH	47.7 (79.7)	56.2 (78.5)	57.6 (82.2)
2nd	EPI	17.9 (7.9)	17.7 (5.6)	14.5 (3.6)
	SCL	29.5 (11.6)	24.8 (17.7)	20.5 (10.3)
	VB	10.1 (3.3)	9.7 (5.3)	7.6 (5.5)
	PITH	42.5 (77.7)	47.8 (71.5)	57.4 (80.6)

(); Proportions of area for the tissue fractions to the total area on the cross-cut section of the internode.