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Abstract : The relationship between chemical components of pod shell and pod dehiscence was investigated using 25 soybean cultivars; 16 with easily dehiscing pods (susceptible cultivars) and 9 with hardly dehiscing pods (resistant cultivars). After air-drying for about three weeks, the pod shells were ground and analyzed for the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemi-cellulose (HCe), cellulose (Ce), uronic acid and calcium. The correlation of the contents of chemical components with the percentage of pod dehiscence (%PD) was examined by principal component analysis. The first principal ingredient score was given by the formula; score $= -0.421$[ADF] -0.038[ADL] $+0.821$[HCe] -0.382[Ce] $+20.556$, where, [ADF], [ADL], [HCe] and [Ce] are percentage of each component in dried pod shell. This score gave an eigenvalue of 30.2 and contribution rate of 97.1%, and the score was higher in the susceptible cultivars than in the resistant cultivars on the average. The multiple regression analysis of the relationship between %PD and the content of chemical components also showed that %PD was best predicted by the regression equation with two chemical components, [HCe] and [Ce]. Water retention capacity and cellulose crystallinity of the pod shell were less different between the susceptible and resistant cultivars. The results in this study suggested that the chemical analysis of dry pod shell may provide useful information on breeding and selection of the resistant cultivars.

Key words : Acid detergent fiber, Acid detergent lignin, Cellulose, Chemical component, Hemi-cellulose, Neutral detergent fiber, Pod dehiscence, Soybean.

Pod dehiscence is mainly affected by moisture content of pod. In both soybean and birdsfoot trefoil, most pods containing more than 10% moisture did not shatter, and those with less than 10% moisture shattered (Metcalfe et al., 1957; Caviness, 1965; Tsuchiya and Sunada, 1977; Tsuchiya, 1987; Romkaew and Umezaki, 2006). Inconsistency may be caused by the difference in the measuring condition especially in relative humidity of 15 or 20% (Anderson, 1955; Metcalfe et al., 1957; Caviness, 1965; Grant, 1996; Tukamuhabwa et al., 2002). The percentage of pod dehiscence (%PD) also varied with the physical or chemical characteristics. As the pod shell desiccates, the exocarp and mesocarp shrink, and the valve of the pod at the septum is separated (dehiscence) due to the tension given to the endoscarp (Spence et al., 1996). The dehiscence may be caused by the tension, which can be associated with the difference in chemical component and/or structure of pod shell.

Thus, it is necessary to examine the relationship between the chemical component and pod dehiscence, and to examine the content of the related chemical components in the pod shell. However, there has been very little effort to study the relationship between the chemical component and pod dehiscence in soybean.

In this study, the chemical components, crystallinity of cellulose and water retention (i.e., water-holding capacity) of soybean pod were measured in 25 soybean cultivars to determine the effect of chemical components and some related properties on pod dehiscence.

Materials and Methods

1. Preparation of plants for analysis

Twenty-five cultivars of soybean (*Glycine max* (L.) Merrill) (see Table 3) were sown in the experimental field of Mie University (Tsu City, Japan) on July 3, 2004. Three seeds per hill were sown at 20 cm spacing in a row approximately 6 m long with 70 cm row spacing. Two or three rows per cultivar were prepared, and the seedlings were thinned to one seedling per hill at two weeks after sowing. Compound fertilizer (N : P_2O_5 : $K_2O = 3 : 10 : 10$) at 100 g m^{-2} and $CaCO_3$ at 100 g m^{-2} were applied as basal dressing. The pod samples were harvested when the pods became a mature color, brown or black. After air-drying for about three weeks,

Table 1. The chemical components (cell-wall components) of pod shells in susceptible and resistant cultivars of soybean.

Cultivars	Chemical components (%)						
	NDF	ADF	ADL	Hemi-cellulose	Cellulose	Uronic acid	Ca
Susceptible cultivars[a]	46.36	37.15	8.50	9.21	28.65	21.06	1.55
Resistant cultivars[b]	44.36	38.32	8.93	6.08	29.39	20.74	1.51
Significance	**	*	*ns*	*	*ns*	*ns*	*ns*
P value	*0.002*	*0.047*	*0.089*	*0.011*	*0.193*	*0.734*	*0.755*

[a] Susceptible cultivars : Akisengoku, Akishirome, Chadaizu, Fukuyutaka, Himeshirazu, Kodane, Kosamame, Kosuzu, Nabeshima, Nattoshoryu, Shirotae, Ootsuru, Sachiyutaka, Tamahomare, Tanbaguro and NS1.
[b] Resistant cultivars : Suzuotome, CM2, CM3, CM4, CM60, SJ4, SJ5, SK1, SK2.
Susceptible and resistant cultivars were clarified in the previous paper (Romkaew and Umezaki, 2006).
ADF, acid detergent fiber; ADL, acid detergent lignin; NDF, neutral detergent fiber.
*,** : indicates significant difference at 0.05 and 0.01 probability levels, respectively.
ns : not significant.

the pod shells were ground with a CYCLOTEC to measure chemical components, water retention (water-holding capacity) and cellulose crystallinity.

These pod samples were the same as those described previously (Romkaew and Umezaki, 2006).

2. Determination of chemical components

Air-dried pod shells were analyzed for the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose (HCe), cellulose (Ce), uronic acid (UA) and calcium (Ca). The contents were shown by the percentage to the dry weight of pod shell, and are respectively represented by [NDF], [ADF], [ADL], [HCe], [Ce], [UA] and [Ca], in this paper. [NDF] and [ADF] were determined according to the method of Van Soest et al. (1991) without the use of sodium sulfite and α-amylase. [ADL] was determined using 72% H_2SO_4 solution as modified by Van Soest et al. (1991). [HCe] and [Ce] were estimated by subtracting [ADF] from [NDF] and [ADL] from [ADF], respectively. [UA] was measured by the method of Blumenkrantz and Asboe-Hansen (1973) with some modification using spectrophotometer at 520 nm, and [Ca] by atomic absorption spectrophotometry (AAS).

3. Determination of water retention and cellulose crystallinity

Water retention (g H_2O dry matter) of the pod shells was determined using the powdered pod shell with less than 80 μm particle size. The values were expressed as the amount of water retained after soaking into 10 ml distilled water for 1 hr at room temperature. The soaked sample was transferred into a plastic container, which has many pinholes on the bottom with a circle filter paper to prevent escape of plant materials. Then, it was put into 15-ml centrifugation tube and centrifuged at 3,000 rpm for 20 min. The container plus the material was weighed and dried to determine the amount of moisture retained.

The crystalline intensity of cellulose in the fine powder of pod shell was evaluated using a Miniflex X-ray diffractometer (Rigaku denki Co. Ltd., Tokyo, Japan). The X-ray diffraction was operated at 10 mA and 30 kV and scanned with a diffraction angle (2θ) ranging from 5° to 35°.

Results and Discussion

One of the major factors leading to a marked yield loss in soybean was pod dehiscence or pod shattering in harvesting. To classify the degree of pod dehiscence in soybean, we used the desiccator method developed by Romkaew and Umezaki (2006). Thirty pods, each containing two seeds, were harvested with three replications, and they were placed in a desiccator cabinet with silica gel at room temperature. Degree of pod dehiscence was recorded at 3, 5, 7, 14, 21, 28 and 35 days after placing in the desiccator (DAD). In consequence, the soybean cultivars were separated into two groups, one with easily dehiscing pods and the other with poorly dehiscing pods, based on the dehiscing percentage of the pods with 10% moisture content (Romkaew and Umezaki, 2006). Here we refer to them as susceptible and resistant cultivars, respectively. Table 1 shows the content of each chemical component in pod shells of susceptible and resistant cultivars (Romkaew and Umezaki, 2006). A significant difference between the two groups was observed in [NDF] (P<0.01), [ADF] (P<0.05) and [HCe] (P<0.05). No significant differences were found in [ADL], [Ce], [UA] and [Ca] between the susceptible and resistant cultivars (Table 1).

The relationship between each of the chemical compositions and %PD was analyzed for 25 cultivars including both susceptible and resistant cultivars by simple linear regression analysis. The numerical values of %PD led by the previous paper (Romkaew and Umezaki, 2006) was used. Table 2 shows the coefficient of correlation between the content (%) of each chemical component and %PD at 3, 5, 7, 14,

Table 2. Coefficients of correlation between the content (%) of chemical components of pod shell and the percentage of pod dehiscence (%PD) at 3, 5, 7, 14, 21 and 35 days after placing in desiccator (DAD) in 25 soybean cultivars.

Chemical components	Correlation of coefficients						
	3DAD	5DAD	7DAD	14DAD	21DAD	28DAD	35DAD
NDF(%)	−0.274ns	−0.110ns	−0.093ns	0.206ns	0.261ns	0.266ns	0.294ns
ADF(%)	0.479*	0.265ns	0.213ns	−0.082ns	−0.151ns	−0.152ns	−0.175ns
ADL(%)	−0.151ns	−0.180ns	−0.224ns	−0.365ns	−0.417*	−0.403*	−0.368ns
Hemi-cellulose(%)	−0.388ns	−0.193ns	−0.157ns	0.149ns	0.213ns	0.216ns	0.242ns
Cellulose(%)	0.550**	0.329ns	0.284ns	0.005ns	−0.056ns	−0.060ns	−0.094ns
Uronic acid(%)	−0.142ns	0.058ns	0.074ns	0.042ns	0.055ns	0.048ns	0.006ns
Calcium(%)	−0.219ns	−0.233ns	−0.300ns	−0.039ns	0.002ns	0.017ns	0.051ns
Water retention (g g^{-1} DM)	−0.126ns	0.002ns	0.077ns	0.134ns	0.147ns	0.150ns	0.155ns
Cellulose-crystallinity	0.219ns	0.067ns	0.030ns	0.188ns	0.195ns	0.199ns	0.299ns

The percentage of pod dehiscence (%PD) was reported in the previous paper (Romkaew and Umezaki, 2006).
*,**: indicates significant difference at 0.05 and 0.01 probability levels, respectively.
ns: not significant.

21, 28 and 35 days after placing in desiccator (DAD). The %PD at 3 DAD was positively correlated with [Ce] (r=0.550, P<0.01) and [ADF] (r=0.479, P<0.05) and negatively with [HCe] (r=0.388, P<0.08). In addition, %PD at 21 and 28 DAD was negatively correlated with [ADL] (r=0.417 and 0.403, respectively, P<0.05). However, these values varied with DAD, and, it is difficult to find some specific chemical components that affect the mechanism of the pod dehiscence of soybeans.

We analyzed the relationship between the combination of several chemical components and the characteristic of pod dehiscence using principal component analysis. We combined the four components, ADF, ADL, HCe and Ce, based on the results shown in Table 2, and calculated the first principal ingredient score as follows: score=−0.421[ADF]−0.038[ADL]+0.821[HCe]−0.382[Ce]+20.556. This equation gave the eigenvalue 30.2 and contribution rate 97.1%. Table 3 shows the score of each cultivar. Comparison by non-parametric test showed that the averaged scores of susceptible cultivars was significantly (P<0.05) higher than that of the resistant cultivars. The first principal ingredient score would be useful for prediction of pod dehiscence in breeding and production.

To predict %PD from the contents of plural chemical components, we selected four combinations of chemical components and obtained multiple regression equations shown in Table 4. A significant correlation was observed between Y (predicted %PD) and %PD at 35 DAD (R=0.443, P<0.05) in the equation with [HCe] and [Ce]: Y=−803.77+14.13[HCe]+26.04[Ce]. However, no significant correlation was observed in other equations shown in Table 4.

Since the moisture contents of pod in the desiccator and %PD changed with the time after harvest, we

Table 3. The first principal ingredient score of each cultivar calculated by principal component analysis.

Susceptible group		Resistant group	
Cultivars	score	Cultivars	score
Akisengoku	9.138	Suzuotome	−5.043
Akishirome	2.834	CM2	−7.073
Chadaizu	−1.155	CM3	1.422
Fukuyutaka	2.666	CM4	−2.571
Himeshirazu	−0.620	CM60	−0.773
Kodane	0.993	SJ4	0.221
Kosamame	1.743	SJ5	2.364
Kosuzu	−17.989	SK1	−4.868
Nabeshima	−1.417	SK2	−3.048
Nattoshoryu	0.950		
Shirotae	10.727		
Ootsuru	4.452		
Sachiyutaka	3.056		
Tamahomare	0.001		
Tanbaguro	6.970		
NS1	−2.980		
AVG	1.210 ^{a}		−2.150 ^{b}

Data with the same letters are not significantly difference at the 5% level, as determined by non-parametric test.
Score=−0.421[ADF]−0.038[ADL]+0.821[HCe]−0.382[Ce]+20.556
ADF, acid detergent fiber; ADL, acid detergent lignin; HCe, hemi-cellulose; Ce, cellulose.

calculated the partial regression coefficients and multiple correlation coefficients in the above equation of [HCe] and [Ce] with %PD at different DAD (Table 5). Partial regression coefficient of [HCe] for %PD at 3 and 5 DAD, and multiple correlation coefficient

Table 4. Multiple regression equations of the contents of plural chemical components with the percentage of pod dehiscence (%PD) at 35 DAD.

Equation	Coefficient (R)
Y=−1106.87+17.44 (NDF)+14.55 (ADF)−19.91 (ADL)	0.409ns
Y=−1106.87+12.08 (ADL)+17.44 (Hemi-cellulose)+31.99 (Cellulose)	0.409ns
Y=−1106.87+12.08 (ADF)+17.44 (Hemi-cellulose)+19.91 (Cellulose)	0.409ns
Y=−803.77+14.13 (Hemi-cellulose)+26.04 (Cellulose)	0.443*

Y: predicted %PD.
The percentage of pod dehiscence (%PD) was reported in the previous paper (Romkaew and Umezaki, 2006).
ADF, acid detergent fiber; ADL, acid detergent lignin; NDF, neutral detergent fiber.
*: indicates significant difference at 0.05 probability levels.
ns: not significant.

Table 5. Partial regression coefficient and multiple correlation coefficient in multiple regression equations of the contents of chemical components with %PD at 3, 5, 7, 14, 21, 28 and 35 DAD.

Days after pacing in desiccator	Partial regression coefficient [a]			Multiple correlation coefficient (R)
	Hemi-cellulose	Cellulose	Constant	
3	6.17ns	19.82**	−608.23*	0.588**
5	8.82ns	22.35*	−688.67*	0.363ns
7	10.77*	25.32*	−785.25*	0.395ns
14	14.18**	27.70*	−857.57*	0.468*
21	14.82**	27.97*	−867.84*	0.489*
28	14.79**	27.72*	−859.07*	0.477*
35	14.13*	26.04*	−803.77*	0.443*

[a] Y (predicted %PD)=−803.77+14.13[HCe]+26.04[Ce]
The percentage of pod dehiscence (%PD) was reported in the previous paper (Romkaew and Umezaki, 2006).
HCe, hemi-cellulose; Ce, cellulose.
*,**: indicates significant difference at 0.05 and 0.01 probability levels, respectively.
ns: not significant.

Table 6. Crystalline/amorphous regions of cellulose, and water retention of pod shell in susceptible and resistant cultivars.

Cultivars	Crystalline-amorphous regions		Ac/(Ac+Aa)	Water retention H_2O g g^{-1}DM
	Ac (cm^2)	Aa (cm^2)		
Susceptible	1.89±0.20	6.20±0.68	0.23±0.02	2.45±0.18
Resistant	1.90±0.17	6.70±0.73	0.22±0.02	2.38±0.26

The values represent the mean±S.E.; n=3.

with %PD at 5 and 7 DAD did not show a significant correlation, but they showed a significant correlation with %PD at other DAD. The partial regression coefficient of [Ce] and a constant showed significant correlation with %PD at all DAD examined.

Meakin and Roberts (1990) proposed that the ultrastructural modification may be initiated by the onset of lignification within the replum and may be ultimately associated with the onset of pod senescence. Yang et al. (1990) concluded that pod dehiscence was associated with the degree of mesocarp lignification. Child et al. (1998) also reported that increased lignification in the dehiscence zone appeared to increase pod dehiscence.

Table 6 shows the crystalline and amorphous regions, and water retention of pod shells in susceptible and resistant cultivars. The crystalline region (Ac) and amorphous region (Aa) of cellulose and the ratio of Ac/(Ac+Aa) were not significantly different between susceptible and resistant cultivars, and neither was water retention (Table 6). The structure of cellulose was long and rigid, and cellulose fibrils were not different among soybean cultivars (data not shown). Pod dehiscence is a phenomenon of hygroscopic movement occurred by the difference in the physical force in the exocarp and endocarp. The bending movement occurs in vertical to the

arrangement of the cuticular fibrous cell layer in the endocarp, namely to the diagonal direction to the inner suture (Nagata, 1973). It is necessary to consider not only the characteristic of cellulose but also the physical and chemical organization of cellulose and some chemical components.

In conclusion, the first principal ingredient score of chemical components in pod shell was useful to distinguish between susceptible and resistant cultivars. Among the multiple regression equations between the contents of chemical components in dried pod shell and %PD at 35 DAD, the equation with [HCe] and [Ce] was the best fit. These approaches may be helpful for developing new cultivars and for preventing loss of soybean yield.

Acknowledgment

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