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Uptake and transport of nutrients and transverse cracking of bean cotyledons

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Abstract

Calcium, Potassium and Magnesium in cotyledon cell walls were negatively correlated with cultivar susceptibility to transverse cracking of cotyledons (TVC). Plant feeding with calcium only slightly changed the percentage of TVC, and Ca, K and Mg contents in the cotyledon cell walls. Susceptibility to TVC was connected with the causes inherent in the root or shoot. Some disturbances in the displacement of Ca and K through the peduncles and K through the petioles in susceptible cultivars were noticed. These cultivars also immobilized a large quantity of Mg in stems, and in this connection they actually had less Mg in the fruit walls, seeds, stems and blades than resistant cultivars.

INTRODUCTION

Poor seed quality in beans has long been a major problem. Transverse cracking of bean cotyledons, appearing only occasionally at the beginning of our century, now occurs very often, seriously lowering the value of bean sowing material many times up to 90%. This is the reason why it has became a serious economic problem.

Pollock (1970) suggested that TVC might be caused by physiological breakage rather than by mechanical impacts. McCollum (1953), Dickson, Duczmal and Shannon (1973) showed that cultivar differences in TVC were associated with seed coat permeability and that susceptible cultivars imbibed water rapidly. Morris (1970) found that structural weakness appeared to be in the cell wall and not in the middle lamella, as rupture occured across the cell walls.

Shannon (1967) found that hypocotyl necrosis, a germination disorder, was associated with low Ca in the seed of some cultivars. Calcium concentration in seeds was quite low compared with that in other parts

of the plant. Snyder (1936) reported 2.47% Ca in the seed coat and only 0.037% in the cotyledons of "Great Northern" bean. Eschandi, Chase and Massey (1970) found that Ca was associated with cellulose and hemicellulose in carrot root cell walls and was believed to be a factor in cell wall rigidity. The presence of Ca in the cell is a cause of selective uptake and transport of many antagonistic ions. Dickson, Duczmal and Shannon (1973) reported that Potassium and Magnesium in cotyledons were negatively correlated with cultivar susceptibility to TVC. They also found that Calcium was sometimes significantly correlated with TVC in seed in a given location, but the correlations could be lacking or of opposite sign in seed grown elsewhere. The known variability of Ca in bean seeds and its association with structural components (Bonner 1936, Echandi et al., 1970) made it desirable to determine if there was any association between TVC and the elemental composition of beans.

MATERIAL AND METHODS

Seed composition. Three different sets of analyses were made for Ca, Mg, K and Na on various seed lots. In the first set, seed of 8 cultivars or inbred lines were analyzed, and in the second set, 11 cultivars were evaluated in duplicate. In both cases samples were selected to cover a wide range of TVC susceptibility. Seed used for the first two sets of analyses were obtained from various sources. For the third test, 22 lines of commercial cultivar Wiejska were grown in pots on sandy soil in a greenhouse. Seeds from every line were tested separately for TVC and analyzed for mineral composition of the cotyledonal cell walls.

Ca — uptake. Ca uptake was tested in three pot experiments. In the first one Ca was introduced into the bed in the form of $CaSo_4 \cdot 2\ H_2O$ in the amount ranging from 0 to 500 mg (CaO) pot. Two potassium levels were used: 0,6 g and 1,2 g K_2O. In the second one the bed reagent was being changed from 5 to 8 pH and the boron level was differentiated. It was administered in two doses (0,02 and 0,06 g/pot of borax). In the last one Ca was introduced through leaves by spraying the plants with 0,5% $CaCl_2 \cdot 6\ H_2O$ several times. In all the experiments the plants were given standard fertilization in the amounts given below (calculated per pot): not including borax in the experiment 2, and potassium in the experiment 1:

0,3 g N in the form of NH_4NO_3, 0,6 g P_2O_5 in the form of $NaH_2PO_4 \cdot 7\ H_2O$, 0,6 g K_2O in the form of KCl, 0,2 g $MgSo_4 \cdot 7\ H_2O$, 0,05 g $Na_2B_4O_7 \cdot 10\ H_2O$, 0,02 g $FeC_6H_5O_7 \cdot 3\ H_2O$, 0,02 g $CuSO_4 \cdot 5\ H_2O$ and 0,001 g $(NH_4)_2 MoO_4 \cdot 2\ H_2O$. The pots were filled with finegrained quartz sand, and the moisture was kept on the level of 60% full water capacity. All the combinations were done four times.

Grafting Experiments. Two cultivars of french bean and two of "dry bean" were used for grafting. In both groups, one cultivar was susceptible and the other resistant to TVC. Grafting was carried out in the following way: in one case the susceptible cultivar was the stock, and the resistant one — the scion; while in the other case — the resistant cultivar was the stock and the susceptible one the scion. The control plants were also grafted, the stock and the scion being of the same cultivar. Grafting was done on the node from which the first leaves shoot up. Ten grafted plants constituted each combination.

Transport of Ca, K and Mg. Investigations on Ca, K and Mg displacement were carried out on three cultivars of french bean, susceptible to TVC: Tendercrop, Saxanova, Improved Landreth, and 3 resistant cultivars: Earliwax, Improved Higrade and Tenderlong. Ten plants, 20 and 30 days after blooming were chosen for analysis from the experimental field in Baranowo near Poznań.

The plants were divided into 7 parts: upper and lower stem, peduncle, fruit wall, seed, petiole and blade. Each analysis was carried out twice.

TVC — test. To evaluate transverse cracking of cotyledons the seed coat was peeled off and the embryo soaked in water for ten hours. TVC in this study is defined as any visible external cracking on the inside or the outside of the cotyledon. Degrees of cracking were not recorded.

Isolating the cell walls and determination of Ca, K and Mg. The method used in isolating the cell walls was a modification of that outlined by Echandi (1970: a 10 g sample of cotyledons was ground and homogenized in 300 ml of glycerol. The homogenate was then centrifuged and the residue resuspended in 300 ml of glycerol, places in an omnimixer for 5 minutes, and then recentrifuged. This was repeated twice. The residue was then resuspended in 100 ml of glycerol and stirred vigorously by hand and filtered through sharakskin filter paper with suction. The filtrates were washed twice with 500 ml of absolute ethanol, once with acetone, and once with ether. The residue was then dried at 60°C.

Ca, K, Na and Mg level were determined by emission and atomic absorptionspectrophotometry of dry ashed samples immersed in 2N HCl solution.

RESULTS

Seed composition. Susceptibility to TVC, percentage of cell wall material, and Ca and Mg content of various seed parts of snap bean lines are presented in Tables 1 and 2. The cultivar Red Kidney, although being a dry bean type, was included since it was known to be resistant to TVC. For this reason we have given the linear correlation coefficients

Table 1

Relation of TVC to Ca in seed and in cell wall material extracted from cotyledons

Cultivars	TVC %	Call walls %	Ca as % dry wt or mg/100 seeds					Cell wall Ca as % cotyledon Ca
			Seed		Seed coats mg	cotyled. mg	cell walls mg	
			%	mg				
Red Kidney	3	24	.093	42.50	29.2	13.4	2.4	18
WB 6-5	20	31	.183	49.66	36.6	13.1	3.0	22
Kinghorn W.	40	28	.177	48.53	36.1	12.4	2.8	22
X-11	57	27	.173	47.38	37.5	9.9	2.0	20
Midas	58	27	.252	48.29	39.5	9.8	1.4	18
H-149	85	22	.148	39.73	29.8	9.9	3.0	4
BBL-274	92	22	.183	37.15	27.9	9.3	.4	4
Early Gella.	94	23	.109	34.16	26.6	7.6	.3	4
Linear correlation of TVC vs. composition								
All 8 cultivars		−.62	−.20		−.42	−.40	−.91*	−.90*	−.83*
Exclud. Red Kidney		−.98**	−.24		−.82*	−.81*	−.88**	−.98**	−.93**

*, ** Significant at 5% and 1% levels, respectively.

Table 2

Relation of TVC to Mg in seed and in cell wall material extracted from cotyledons

Cultivar	TVC %	Call walls %	Mg as % dry wt or mg./100 seeds					Cell wall Mg as % cotyledon Mg
			Seed		Seed coat mg	cotyl. mg	cell walls mg	
			%	mg				
Red Kidney	3	24	.132	48.87	7.7	40	1.6	4
WB 6-5	20	31	.199	54.10	9.5	45	1.5	3
Kinghorn W.	40	28	.191	52.03	9.8	52	1.4	3
X-11	57	27	.178	48.78	9.3	40	1.3	4
Midas	58	27	.182	34.97	5.2	30	1.0	3
H-149	85	22	.159	42.79	9.0	34	.9	2
BBL-274	92	22	.173	35.32	7.5	28	.8	3
Early Galla.	92	23	.132	41.23	9.8	31	.7	2
Linear correlation coefficients of TVC vs composition								
All 8 cultivars		−.62	−.71		−.65	.02	−.68	−.96**	−.67
Exclud. Red Kidney		−.98**	−.81**		−.78*	−.14	−.79*	−.95**	−.50

*, ** Significant at 5% and 1% levels, respectively.

relating TVC to Ca and Mg, both for all 8 cultivars and for the 7 remaining after excluding "Red Kidney" from the analyses.

When "Red Kidney" was excluded in the comparisons there was a marked increase in the correlation coefficients for most factors studied.

This was especially true of the relation of TVC to the amount of cell wall material extracted from the cotyledons. The highly significant negative correlation would indicate that TVC resistance within these snap bean lines was due to thicker cell walls or to smaller cells, either of which would result in more cell wall material.

The relation between TVC and concentration of Ca in seed was not significant, but when Ca was calculated on the basis of mg per 100 seeds, the correlations between TVC and seed coat Ca improved. Especially good correlation was found between TVC and the amount of Ca associated with cotyledons, cotyledonal cell walls and cell walls relative to total cotyledonal Ca.

When "Red Kidney" is omitted, the correlation between conc. of Mg in the seed and TVC, and the correlation of mg Mg per 100 seeds with TVC were both significant. The correlation of TVC to Mg — content in cell walls of 100 seeds was also significant, but there was little significance in the relationship between TVC and the percentage of total Mg associated with cell walls.

These data suggest that resistance to TVC in these snap bean lines is a function of the amount of cell wall material in the cotyledons, and that Ca and Mg are also associated with resistance. However, the second lot of 11 cultivars, tested as shown in Table 3, did not support this hypothesis. The amount of cell wall material was much more variable and due to the difficulty in performing the extraction with bean

Table 3

Relation of TVC to Ca and Mg in cell walls extracted from cotyledons

Cultivar	TVC %	Cell walls %	Ca as % dry wt of cell walls	Mg as % dry wt of cell walls
Spartan Arrow	5	28.6	.019	.096
Earliwax	12	25.7	.015	.051
WB 6-5	20	28.9	.014	.058
Avalanche	32	21.9	.014	.061
Slimgreen	50	32.3	.018	.049
Astro	50	31.0	.012	.045
Orbit	54	17.8	.013	.041
W 26	57	17.2	.013	.030
OSU 58	74	20.6	.011	.057
Sprite	77	23.3	.019	.108
Tenderette	94	32.1	.009	.034
Linear correlation coefficients of TVC vs composition				
All cultivars		−.10	−.40	−.11
Excluding Sprite		−.06	−.74*	−.69*

* Significant at the 5% level.

cotyledons, the method is probably only semi-quantitative. The cultivar Sprite was most unusual in having high TVC and also high Ca and Mg in cotyledonal cell walls. By eliminating "Sprite" from the comparison, a significant negative correlation between TVC and cotyledonal cell wall Ca and Mg was obtained.

In order to avoid differences in sources and handling, 22 lines of Cultivar Wiejska were grown in sandy soil in pots and were handled similarly before testing. The results are summarized in Table 4.

Table 4

Relation of TVC to Ca, K, Mg and Na content in cell walls extracted from cotyledons of 22 lines of cultivar Wiejska

TVC / Cation (% dry wt)	<80%	81—90%	>90%	Linear correlation coefficients of TVC vs composition
Ca	0.397	0.368	0.353	—.049*
K	0.749	0.684	0.651	—0.80**
Ca+K	1.146	1.039	1.005	—0.68**
Mg	0.127	0.130	0.127	—0.23
Na	0.035	0.030	0.027	—0.21

*, ** Significant at 5% and 1% levels, respectively.

There was a highly significant negative correlation between TVC and the percentage of K and Ca+K in the cotyledonal cell walls, and a significant negative correlation between TVC and the percentage of Ca in cotyledonal cell walls. In contrast to the previous seed lots there was a low and not significant correlation between TVC and the cotyledonal cell wall percentage of Mg.

Ca — u p t a k e. The susceptibility to TVC and Ca, K and Mg contents in the cell wells of cultivar Wiejska in correlation with feeding calcium are given in Tables 5, 6 and 7. Neither small nor larger $CaSO_4$ contents in the bed were reported to influence the change in susceptibility to TVC or the contents of Ca, K an Mg. While spraying the plants with 0,5 % $CaCl_2$ significantly decreased susceptibility to TVC and increased the contents of Ca and K in the cell wall.

G r a f t i n g e x p e r i m e n t s. Susceptibility to TVC of plants originating from grafted ones is presented in Table 8. In the cases of two cultivars, Wiejska and Krakowska, the resistance of TVC in grafted plants depended on the scion. When Krakowska cultivar constituted the scion, the number of TVC amounted to 58, that is, it was similar to the results obtained with Krakowska cultivar. When Wiejska cultivar constituted the scion, the TVC percentage was very close to the TVC of Wiejska cultivar. In the next pair of cultivars — Tenderwhite and Ten-

dercrop, the TVC percentage in grafted plants depended on the stock. When Tendercrop was used as the stock, the TVC percentage was 56, that is it was similar to that of Tendercrop cultivar. Using Tenderwhite as the stock, the result obtained was similar to that of Tenderwhite cultivar.

Transport of Ca, K and Mg. Both cultivars, susceptible and resistant, had similar Ca contents in stems and leaves. The susceptible cultivar accumulated a large quantity of Ca in the peduncle, and in this

Table 5

Relation of Ca and K in the sand to TVC and Ca, K and Mg in cell walls extracted from cotyledons of cultivar Wiejska

CaO (mg)	TVC[z] %	Ca of cell walls %	K of cell walls %	Mg of cell walls %
		0.6 g K_2O		
0	86	0.390	0.625	0.033
100	81	0.415	0.665	0.035
200	85	0.435	0.635	0.032
300	92	0.365	0.670	0.021
500	94	0.355	0.585	0.032
		1.2 g K_2O		
0	80	0.435	0.760	0.136
100	84	0.310	0.680	0.126
200	88	0.355	0.795	0.155
300	96	0.395	0.720	0.153
500	80	0.400	0.720	0.150

[z] Significant at 5% level.

Table 6

Relation of pH and B in the bed to TVC and Ca, K and Mg in cell walls extracted from cotyledons of cultivar Wiejska

pH	TVC[z] %	Ca of cell walls %	K of cell walls %	Mg of cell walls %
		0.02 g borax		
7	93	0.335	0.570	0.105
6	99	0.325	0.640	0.101
7	91	0.281	0.680	0.122
8	92	0.265	0.590	0.108
		0.06 g borax		
5	80	0.325	0.675	0.099
6	84	0.305	0.705	0.114
7	99	0.225	0.565	0.108
8	79	0.365	0.690	0.114

[z] — Significant at 5% level.

Table 7

Relation of Ca given to the plants to TVC and Ca, K and Mg in cell walls extracted from cotyledons of cultivar Wiejska

	TVC[z] %	Ca of cell walls[z] %	K of cell walls %	Mg of cell walls %
$CaCl_2$ spraying	75	0.460	0.900	0.137
Without spraying	85	0.432	0.732	0.154

[z] Significant at the 5% level.

Table 8

Transverse cracking of cotyledons from grafted plants of some bean cultivars

scion stock	TVC[z] %	scion stock	TVC[z] %
Wiejska Wiejska	92	**Tenderwhite** Tenderwhite	86
Krakowska Krakowska	48	**Tendercrop** Tendercrop	52
Wiejska Krakowska	79	**Tenderwhite** Tendercrop	56
Krakowska Wiejska	58	**Tendercrop** Tenderwhite	77

[z] — Significant at 1% level.

connection they showed Ca contents in the peduncle nearly twice as high as in resistant cultivars. In comparison with resistant cultivars they also had a slightly lower Ca contents in the fruit wall and seeds. Potassium accumulated in the fruit walls and petiole in all cultivars, and in peduncles in the susceptible cultivars.

The susceptible cultivars also showed significantly higher K contents in peduncles and petioles than resistant cultivars. Both susceptible and resistant cultivars also differed in Mg contents. They had only half of the amount of Mg that was present in resistant cultivars in fruit walls, seeds, petioles and blades.

DISCUSSION AND CONCLUSION

Since Morris (1970) observed that the structural weakness in the cotyledons was the cell wall, we tried to determine if there was a relation of the amount of cell wall to TVC, and if the elemental composition of the cotyledons or the cotyledonal cell walls was associated with TVC.

There was a highly significant correlation of cell wall material to TVC in the first test but not in the third one. A better, more reproducible

Table 9

Transport of Ca, K and Mg in susceptible and resistant bean cultivars

Part of the plant	Susceptible cultivars[z]			Resistant cultivars[y]		
	Ca**	K**	Mg**	Ca*	K**	Mg**
	as % dry wt			as % dry wt		
Stem-lower part	1.2	1.6	.131	1.4	1.8	.108
Stem-upper part	1.5	2.1	.079	1.4	1.9	.104
Peduncle	2.6	2.4	.061	1.4	1.8	.049
Fruit wall	1.1	2.5	.064	1.3	2.5	.122
Seed	.7	1.7	.085	.9	1.6	.133
Petiole	2.3	2.8	.036	2.1	2.3	.076
Blade	4.5	1.9	.038	4.5	2.0	.074

[z] — Average of three cultivars: Tendercrop (TVC — 90%), Saxanowa (TVC — 90%) and Improved Landreth (TVC — 91%);

[y] — Average of three cultivars — Earliwax (TVC — 61%), Improved Higrade (TVC — 52%) and Tenderlong (TVC — 36%).

*, ** Significant at 5% and 1% levels, respectively.

quantitative method for extracting cell wall material is needed to establish the importance of cell wall material.

We found a consistent negative correlation between TVC and Ca, K and Mg in the cotyledonal cell walls. Susceptible cultivars had less Ca, K and Mg in cotyledonal cell walls that resistant ones.

Increasing the Ca contents in bed only slightly caused the decrease of TVC number and the increase of concentration of both Ca, and K, and Mg. Spraying the overground portion of the plant with calcium also decreased the TVC percentage more distinctly, but not much, and increased Ca, K and Mg contents in the cell walls of cotyledons. It may be supposed, then, that calcium accessibility in the environment does not play ony significant role, and that the content of these elements in cell wall mainly depends on the possibility of their displacement from root to seeds. Grafting experiments demonstrated that susceptibility to TVC may be dependent on the root, as is the case with Tenderwhite, Tendercrop cultivars or on the shoot as, for example, in case of Wiejska and Krakowska cultivars. The different reaction the two pairs of the cultivars can not be explained on the basis of the experiment because the content of Ca, K and Mg were not analysed in the roots. It suggests the genetically conditioned character of this phenomena and the possibility of modification of the mechanism of the phenomena according to the cultivar differences. It was found that Ca translocation in plants is stopped in the peduncle of susceptible cultivars, where twice as much is accumulated. Similarly K accumulates in larger quantities just in the peduncles of susceptible cultivars. Susceptible cultivars also immobilize large quantities of magnesium in shoots, and therefore its contents in the fruit walls, seeds, petioles and blades is reduced by half as compared

with the stem. Mg contents in the fruit walls, seeds, petioles and blades of susceptible cultivars constitutes hardly half of the amount of Mg that is contained in the same parts of resistant cultivars. It could suggest that bean susceptibility to TVC is connected with the presence of "locus barrier" in the roots and shoots, which makes the translocation of a suitable amount of Ca, K and Mg to the seeds difficult.

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Wpływ pobierania i przemieszczania makroskładników na poprzeczne spękania liścieni fasoli

Streszczenie

Stwierdzono, że zawartość wapnia, potasu i magnezu w ścianach komórkowych była skorelowana z wrażliwością odmian fasoli na poprzeczne spękanie liścieni (TVC).

Żywienie roślin wapniem nieznacznie tylko wpływało na ilość spękanych liścieni i zawartość Ca, K i Mg w ścianach komórkowych liścieni. Wrażliwość na TVC była związana z właściwościami fizjologicznymi pędu. Zauważono zakłócenia w przemieszczaniu Ca i K przez szypułki kwiatowe i K przez ogonki liściowe odmian wrażliwych. Te odmiany unieruchamiały również znaczną ilość Mg w pędach i w związku z tym posiadały istotnie mniej Mg w ścianach owocu, nasienia, ogonkach i blaszkach liściowych niż odmiany odporne.