Effect of Virus Yellows on Guard Cell Chloroplasts in Sugar Beets[1]

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Beet yellows virus causes a deficiency of chloroplasts in cells along the cleared veins of infected beets and in general causes a visible disorganization of chloroplasts [Esau (2)[3]]. Esau also noted inclusion bodies in the stomatal guard cells of infected plants.

From the breeding standpoint, there are no precise selection criteria for resistance to beet yellows virus or to beet western yellows virus. In this study the relationships of disease, genotype, and stomatal guard cell chloroplast condition and number were examined. The study was intended not only as a search for a selection criterion but also to relate chloroplast condition and number to various other characters and thus shed additional light on disease characteristics.

Materials and Methods

The experiment was conducted in the greenhouse at Salinas, California, during the winter of 1964-65. It consisted of 2 populations, 3 treatments, and 10 replications. There were 6 plants (individual pots) per replication, making a total of 60 plants per population within treatment. The two populations were 413B and 5511. The former was developed by five cycles of selection for yellows resistance in US 75. The latter was a yellows susceptible selection from the inbred NB2. In field tests 413B has shown considerable tolerance to yellows (3). The three treatments were: no infection; infection with beet yellows virus (Brawley strain); and an infection of beet western yellows virus combined with an unidentified strain of beet yellows virus.

Six characters were studied:

(1) Chloroplast number per pair of stomatal guard cells
(2) Chloroplast disorganization score (0 = complete disorganization, 5 = no disorganization)
(3) Yellowing score (0 = all leaves very yellow, necrotic and etched, 5 = no yellowing relative to the non-inoculated entry)

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[3] Numbers in parentheses refer to literature cited.

(4) Root weight in grams
(5) Top weight in grams
(6) Total plant weight in grams

The experiment was planted on November 10, 1964, and inoculated on December 8. The degree of yellowing was scored on February 15, 1965. Chloroplast number and disorganization score were determined one replication per week, starting January 11; (hence replication effect was confounded with growth stage effect for these two characters). Five determinations of chloroplast number were made in each plant. Chloroplasts in the guard cells of the noninfected plants reached their highest degree of organization in the most mature leaves, but they did so prior to senescence. Therefore, in determining chloroplast number and condition the most mature leaves were used for sampling. For these determinations, a small piece of epidermis was stripped from the dorsal side of the leaf, mounted on a glass slide in a drop of water, covered with a cover glass and examined under 430X magnification. Using this technique, the chloroplasts in the stomatal guard cells were all visible and easily counted except in those cases where there was a high degree of chloroplast disorganization or degradation.

Root and top weight determinations were made on March 18, but only six replications were used since it was necessary to save plants in the other four replications as inoculum source plants for other studies.

Results

The means for populations, treatments and treatments within populations for all six characters are shown in Table 1. Their least significant differences are also included.

The effect of beet yellows infection (Brawley strain) was more severe for every character (although not significantly so in all cases) than the combined infection. The unidentified strain of beet yellows virus in the combined infection was undoubtedly less virulent than the Brawley strain of beet yellows virus. Hence the combined infection was not as damaging as the Brawley strain by itself.

Under conditions of this experiment, the number of chloroplasts per pair of guard cells appeared to be little influenced by the virus infection. There was, however, a difference between populations and a significant population by treatment interaction. Hence genotype is the principal determinant of chloroplast number.

Table 1.—Means and least significant differences for the six characters of interest.

Population and/or treatment	Chloroplast number	Chloroplast condition	Yellowing score	Root weight (gm)	Top weight (gm)	Total weight (gm)
413B	15.24	2.98	4.59	60.4	39.6	100.0
5511	14.92	2.56	3.74	31.8	36.2	68.0
LSD 0.05	0.27	0.23	0.13	3.6	2.7	5.3
BYV	14.99	2.49	3.44	36.8	33.4	70.2
BYV & BWYV	15.20	2.77	4.07	42.6	37.7	80.3
Check	15.06	3.06	5.00	58.9	42.6	101.5
LSD 0.05	0.33	0.29	0.16	4.4	3.3	6.5
413B						
BYV	14.65	2.88	4.38	57.2	39.3	96.5
BYV & BWYV	15.54	3.03	4.40	54.5	39.3	93.8
Check	15.54	3.03	5.00	69.5	40.1	109.6
LSD 0.05	0.47	0.40	0.23	6.3	4.6	9.2
5511						
BYV	15.31	2.10	2.50	16.4	27.5	43.9
BYV & BWYV	14.85	2.50	3.73	30.7	36.1	66.8
Check	14.59	3.08	5.00	48.3	45.1	93.4
LSD 0.05	0.47	0.40	0.23	6.3	4.6	9.2

In the case of chloroplast condition populations, treatments and their interaction were all significant, with treatments having the greatest effect. The significant interaction results from the fact that chloroplast condition was not affected by treatment in 413B but was affected in 5511. Therefore, yellows virus infection results in more or earlier guard cell chloroplast disorganization in susceptible genotypes than in more tolerant or resistant genotypes.

A significant amount of yellowing resulted from infection; also, infected plants of 5511 were considerably more yellow than those of 413B. The interaction of treatment and genotype indicates that the yellowing caused by a particular virus or strain is partially dependent on the genotype of its host.

Root weight reductions due to infection, significant in both populations, were particularly drastic in 5511 where the Brawley strain of beet yellows virus reduced root yield 66%. The mixed infection caused a 36% reduction. In 413B the yield losses due to infection were not different for the two treatments but were 18 and 22% relative to the check. Top weight of 413B was unaffected by infection, but there was a significant reduction in 5511, 39 and 20% relative to the check. Total weights followed the pattern of root weights, with significant reductions due to infection in both populations.

Table 2.—Simple correlation coefficients calculated from treatment means within populations.

Character	Chloroplast condition	Chloroplast number	Root weight	Top weight
Yellowing score	0.975**	—0.146	0.902*	0.959**
Chloroplast condition		—0.119	0.895*	0.952**
Chloroplast number			0.114	—0.347
Root weight				0.761

Simple correlation coefficients based on population means within treatments are shown in Table 2. Yellowing score is positively correlated with chloroplast condition, root weight and top weight. Chloroplast condition is positively correlated with root and top weight. These correlations resulted from the fact that yellows-free beets were heavier, greener and had a higher degree of chloroplast organization. Chloroplast number was not significantly correlated with any of the other characters.

No cell inclusions, as described by Esau (2), were observed in the guard cells or other epidermal cells. This does not exclude the possibility that inclusions might be observed by using certain staining techniques.

Discussion

The number of chloroplasts in the stomatal guard cells was unaffected by disease treatment but was genotype dependent. Neither was it significantly correlated with any other character. Hence this character would seem to offer no promise as a selection tool or measure of disease resistance.

The degree of chloroplast disorganization was affected by disease treatment, genotype and a treatment by genotype interaction. This genotype and interaction effect, plus the fact that degree of disorganization is a rather poorly defined index, indicates that it would be of little practical value in individual plant selection.

Yellowing score relative to the check appears to be genetically conditioned and correlated with root yield. However, McFarlane and Bennett (3) have shown in extensive field tests with diverse genotypes that there is little correlation between yellowing and yield reduction due to yellows infection.

Yield reductions of both root and top were affected by both treatment and genotype. 413B shows considerable disease tolerance relative to 5511. The correlation of root and top yield with yellowing and chloroplast condition is expected from examination of the means and is a logical relationship. Yellowing and chloroplast degradation would be expected to depress photo-

synthesis and hence plant yield. The reason for chloroplast degradation may be [as Bawden (1) suggests] that cells from plants infected with yellows type viruses contain more starch than normal, and chloroplasts may be so gorged with starch that they burst.

Among the six characters, comparative root yield appears to be the most reliable indicator of disease resistance. However, even this character leaves much to be desired as a selection criterion when selection is being made on an individual plant basis.

Summary

A greenhouse test of two populations inoculated with beet yellows and beet western yellows virus showed that the number of chloroplasts in the stomatal guard cells was unaffected by disease treatment. The degree of chloroplast disorganization and yellowing of the leaves are related to treatment, but neither appears to be of much value in making individual plant selections. Root and top yields are related to yellowing and chloroplast condition and are affected by treatment. None of the six characters studied provide a completely reliable index or measure of disease resistance, but among them comparative root yield is likely to be best.

Literature Cited

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