SCREENING FOR TBV-RESISTANCE IN SEEDLING POPULATIONS OF *Tulipa* L.

Th.P. Straathof, W. Eikelboom, J.M. Van Tuyl
DLO-Centre for Plant Breeding
and Reproduction Research
Wageningen
The Netherlands

D. Peters
Agricultural University Wageningen
Department of Virology
Wageningen
The Netherlands

Abstract

Tulip breaking virus (TBV) threatens bulb and flower production of tulips seriously. Breeding for resistance as a tool to prevent this disease resulted already in a screening test at clonal level and different levels of resistance were detected. Clonal tests are performed under greenhouse conditions after inoculation by viruliferous aphids. By using several bulbs of a genotype an accurate level of resistance can be determined. All cultivars of *T. gesneriana* tested were susceptible, but partial and even absolute resistance was found in *T. fosteriana* cultivars. To introduce resistance from *T. fosteriana* in the *T. gesneriana* assortment interspecific crosses were made. To select TBV resistant hybrids efficiently, a screening test, applicable at individual seedlings, is described.

To test individual seedling selection, an incomplete diallel was made between the susceptible *T. gesneriana* cultivars 'Christmas Marvel', 'Kees Nelis' and 'Lustige Witwe', the partial resistant *T. fosteriana* cultivars 'Juan' and 'Madame Lefeber' and the absolute resistant *T fosteriana* cultivars 'Cantata' and 'Princeps'. Individual seedlings were inoculated using viruliferous aphids in the first, third or fifth year after sowing. Six weeks after inoculation, leaves were tested for TBV by ELISA. All seedlings were grown till maturity and the occurrence of breaking symptoms in the flower was monitored.

Individual resistant seedlings could be selected. However, susceptible plants can occur (escapes) between the selected plants and also resistant plants can be discarded (missings). The efficiency of this seedling test will be discussed.

Key words: breeding, ELISA, inheritance, selection, tulip breaking virus

1. Introduction

Tulip breaking virus (TBV), the causal agent of flower breaking, is one of the most important pathogens in tulip. The virus is transmitted non-persistently by aphids such as *Myzus persicae* and *Macrosiphum euphorbiae* (Hammond & Chastagner, 1989) and can therefore be spread through the field in a short period of time. Within the plant, the virus will be transported from the infection site(s), which will mainly be created on the leaves or stem, to sink areas like the flower and the bulb. TBV causes reduction in bulb yield and flower quality. The virus is controlled by rogueing infected plants, or spraying with mineral oils or synthetic pyrethroid insecticides. These measures are expensive, not completely effective and the input of insecticides has to be reduced because of

environmental protection. Another way of controlling this disease is the use of TBV-resistant cultivars.

To screen plants for TBV-resistance, several tests are developed. Plants can be infected mechanically or with aphids (Romanow *et al.*, 1991; Eikelboom *et al.*, 1992). The latter method is probably more reliable since it approaches the way plants are infected in the field and comprises all components of resistance. The number of aphids, the aphid species, the starvation period and the acquisition time can all influence the transmission of the virus. Furthermore, the infection can be performed at different plant stages (e.g., sprouting, before and during flowering) and at different plant sites (e.g., stem, first, second or third leaf) (Thomson, 1980; Eikelboom *et al.*, 1992).

The infection can be determined by enzyme-linked immunosorbent assay (ELISA) on bulb material (Romanow *et al.*, 1986). Furthermore, planting the bulbs a year after infection will result in leaf and flower symptoms. ELISA can also be performed on leaf and stem tissue. Bulbs and leaves, however, do not always give the same results (Romanow *et al.*, 1991). Wounding the leaf or stem one week before testing by ELISA will result in higher virus concentrations, probably because of a sink-relation or/and wound effect (Eikelboom *et al.*, 1992; Van der Vlugt, 1994).

Resistance within the *T. gesneriana* assortment has thus far not been found. Screening other *Tulipa* species resulted in several genotypes with high levels of resistance to TBV. Specially in *T. fosteriana*, cultivars were found with high or even absolute resistance to TBV (Romanow *et al.*, 1991; Eikelboom *et al.*, 1992). To introduce resistance from *T fosteriana* in the *T. gesneriana* assortment interspecific crosses were made. To select TBV resistant hybrids efficiently, a screening test, applicable to individual seedlings of one to five years old, is described.

2. Materials and Methods

A time schedule of the experiment is presented in Table 1. In 1990, an incomplete diallel was made between the susceptible *T. gesneriana* cultivars 'Christmas Marvel', 'Kees Nelis' and 'Lustige Witwe', the partial resistant *T. fosteriana* cultivars 'Juan' and 'Madame Lefeber' and the absolute resistant *T. fosteriana* cultivars 'Cantata' and 'Princeps'. Seeds of 32 populations (Table 2) were sown in separate pots and placed outside in a gauze tunnel. Per population maximal 300 seeds were used. Because of the different germination percentages per population, completely balanced experiments could not be carried out. Seedlings were divided in three groups and tested in 1991, 1993 (non flowering plants) and 1995 (flowering plants).

In 1991, seedlings were inoculated with TBV in the greenhouse using ten viruliferous aphids of *Myzus persicae* per seedling, when they reached 1/2 - 2/3 of their maximal length. Per population maximal 64 seedlings were tested. Five weeks after inoculation the leaf tip was decapitated (wounding) and one week later a small part of the leaf, just below the wound, was tested by ELISA. Plants with an ELISA value below a significant ELISA threshold value were classified as healthy, while seedlings with an ELISA value above the threshold were classified as diseased. Per population, average ELISA values and percentage healthy plants were calculated and analyzed by ANOVA.

All bulbs were harvested and further cultivated in a gauze tunnel till flowering in 1995. In 1993 and 1995, respectively three and five year old seedlings were used for a comparable experiment as described for 1991. Results of the three seedling tests were compared. Using the percentages of healthy plants, diallel analysis was carried out. The general combining abilities (GCA's) and combined other effects (e.g., specific combining ability, reciprocal and population effects) were calculated.

In 1995, most of the seedlings inoculated in 1991 or 1993 flowered and were evaluated for virus infection by breaking symptoms. Plants with no breaking symptoms were classified as healthy and those with breaking symptoms were classified as diseased. Four groups of plants can be distinguished when the ELISA and colour breaking results are analyzed:

1. ELISA negative in 1991 or 1993 and no colour breaking in 1995: resistant
2. ELISA negative in 1991 or 1993 and colour breaking in 1995: escapes
3. ELISA positive in 1991 or 1993 and no colour breaking in 1995: missings
4. ELISA positive in 1991 or 1993 and colour breaking in 1995: susceptible

The flower breaking of the seedlings inoculated in 1995 will appear in 1996. Plants showing no flower breaking in 1995 (seedlings inoculated in 1991 or 1993) and 1996 (seedlings inoculated in 1995) will be cultivated and retested at clonal level in 1998 (Table 1).

Table 1: Time schedule of the three seedling tests.

Name	Sowing	Inoculation	ELISA leaf	Flower breaking	Clone test
1991	1990	1991	1991 (6 weeks after inoculation)	1995	1998
1993	1990	1993	1993 (6 weeks after inoculation)	1995	1998
1995	1990	1995	1995 (6 weeks after inoculation)	1996	1998

3. Results

In Figure 1, the relation between the average ELISA values and the percentage healthy plants is presented. A significant correlation ($r = 0.84$) was found between both values. Large differences in TBV levels were detected within and between the populations. ANOVA showed highly significant differences ($P < 0.001$) between populations and parents. In Table 2, the percentage of healthy plants per population is given. In general, populations obtained from two resistant parents showed less infected plants than populations obtained from two susceptible parents. Comparison of the results of the seedling test in 1991 with the results of the seedling tests in 1993 and 1995 are presented in Figure 2. A significant correlation was found between the three years.

Diallel analysis gave significant GCA ($P < 0.001$) effects in all three years. GCA values for the 7 parents are given in Table 3 together with the percentage of healthy bulbs determined in a clone test (Eikelboom *et al.*, 1992). Significant correlations in GCA values were found between the three years. In all tests 'Cantata' and 'Princeps' had a significant better GCA value than the other cultivars. The combined other effects were significant in 1991 ($P < 0.001$), although the mean square for GCA effect (83.8; df = 6)

was much larger than the mean square for combined other effects (2.58; df = 15). In 1993 the combined other effects was not significant and in 1995 the combined other effects were significant only at $P < 0.05$. In Figure 3, the percentage of healthy plants per population determined in the seedling test of 1991 is plotted against the percentage of healthy plants calculated for GCA effects only. A high correlation (r = 0.94) was found between the determined and the calculated number of healthy plants. Vertical distances between each point and the line represent deviations from additivity.

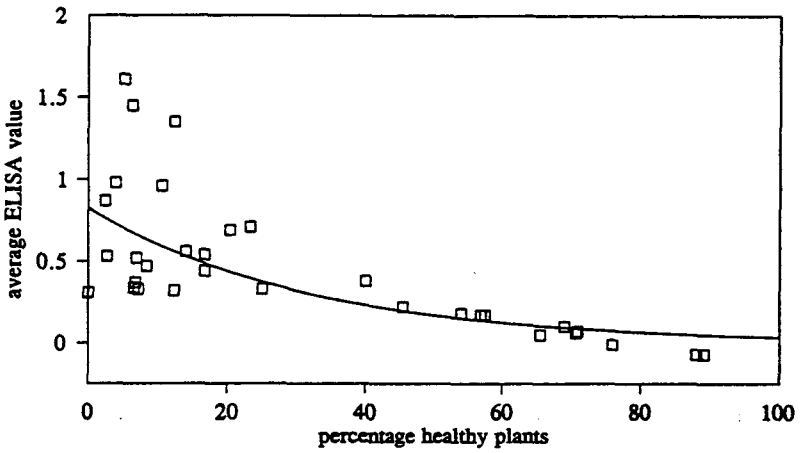

Fig. 1: The correlation between average ELISA values and the percentage of healthy plants of 32 tulip populations after inoculation of seedlings by TBV viruliferous aphids in 1991.

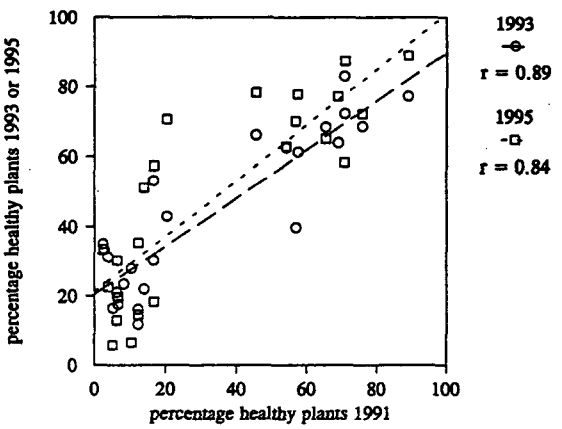

Fig. 2: The correlation between the percentage of healthy plants of 32 tulip populations after inoculation by TBV viruliferous aphids in 1991, 1993 and 1995.

Table 2: Percentage of healthy plants based on ELISA values of 32 tulip populations after inoculation by TBV viruliferous aphids in 1991 (number of seedlings tested).

	Susceptible			Moderate		Resistant		
	CM	KN	LW	JU	ML	CA	PR	Mother
Christmas Marvel	**5.2** (58)	**6.3** (64)	**10.5** (57)	**3.9** (52)	**2.4** (41)	**23.3** (43)	**20.4** (54)	**10.0** (369)
Kees Nelis	**12.3** (57)	**7.1** (56)	**12.3** (64)	**6.9** (29)	**16.7** (42)	**57.5** (47)	**25.0** (20)	**18.7** (315)
Lustige Witwe	**6.7** (60)	**6.5** (62)	**8.3** (36)	**0.0** (13)	**2.7** (37)	**54.1** (37)	**40.0** (30)	**16.0** (275)
Juan					**14.0** (57)	**75.9** (58)	**56.9** (58)	**49.1** (173)
Madame Lefeber				**16.7** (48)		**70.9** (55)	**45.6** (57)	**45.6** (160)
Cantata				**65.5** (55)	**69.0** (58)		**89.1** (55)	**74.4** (168)
Princeps					**70.7** (58)	**88.0** (50)		**78.7** (108)
Father	**8.0** (175)	**6.6** (182)	**10.2** (157)	**24.4** (197)	**33.5** (293)	**63.5** (290)	**49.6** (274)	**32.4** (1568)

On analysing the ELISA values obtained 6 weeks after inoculation and the flower breaking observations in 1995 four groups could be distinguished. In Table 4, the percentages per group are presented for the 32 seedling populations inoculated in 1991. Small deviations between Table 1 and Table 4 are due to the fact that not all plants flowered in 1995 and some seedlings died during cultivation. In the 1993 experiment, similar results were obtained. Of the seedlings tested in 1991, 17.8% were resistant, 64.8% susceptible, while 10.3% were escapes and 7.1% missings. Of the total population, 24.9% (17.8% resistant plants + 7.1% missings) of the seedlings showed to be healthy in 1995. If in 1991 only the healthy plants (28.1% = 17.8% resistant plants + 10.3% escapes) were selected 63.3% (17.8 / [17.8 + 10.3]) of the selected population would be resistant in 1995.

Table 3: General combining ability values (GCA) of 7 tulip cultivars obtained from an incomplete diallel tested at seedling level for TBV resistance in 1991, 1993 or 1995 and corresponding percentage of healthy bulbs determined in a clone test.

	GCA 1991	GCA 1993	GCA 1995	Percentage healthy bulbs[z]
Christmas Marvel	0	0	0	0
Lustige Witwe	9	-3	-2	4
Kees Nelis	10	-6	11	4
Juan	15	-3	12	24
Madame Lefeber	13	7	25	79
Princeps	40	19	43	100
Cantata	55	37	44	100
Grand Mean	-9	27	12	

[z]Eikelboom et al., 1992.

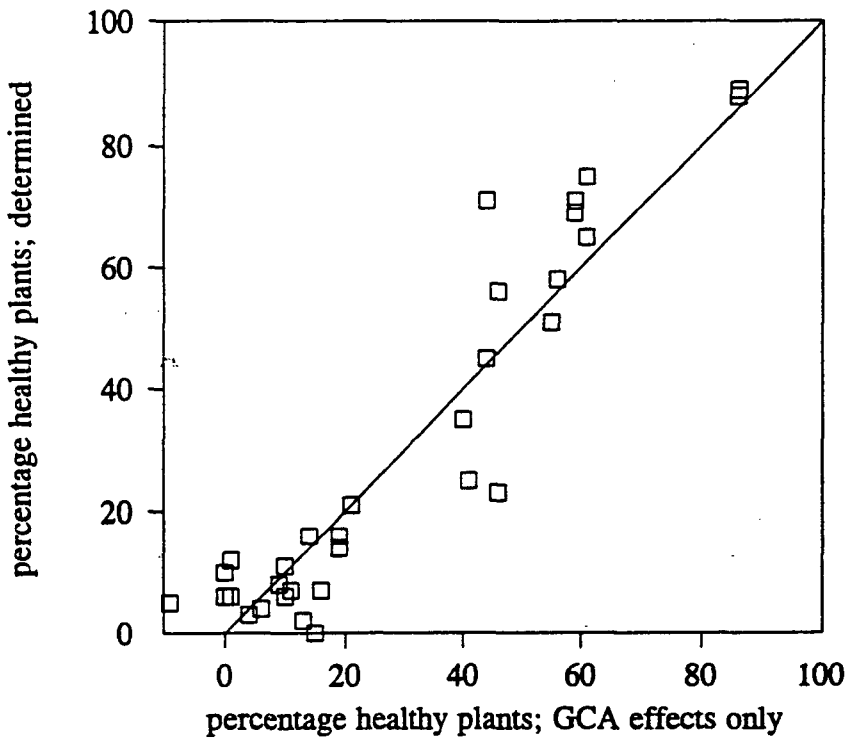

Fig. 3: Relation between percentage of healthy plants of 32 tulip populations determined after inoculation by TBV viruliferous aphids in 1991 and the percentage of healthy plants calculated by GCA effects only.

Table 4: Percentage of resistant plants[w], susceptible plants[x], missings[y] and escapes[z] of 32 tulip populations in 1991 by TBV viruliferous aphids as determined 6 weeks after inoculation by ELISA and flower breaking in 1995.

	Susceptible						Moderate						Resistant			
	CM		KN		LW		JU		ML		CA		PR	
CM	0.0[w]	89.3[x]	1.6	92.0	0.0	86.8	0.0	82.0	0.0	73.0	9.1	3.6	9.7	77.4
	5.4[y]	5.4[z]	4.8	1.6	1.9	11.3	14.0	4.0	27.0	0.0	18.2	9.1	9.7	3.2
KN	3.7	85.2	0.0	91.3	0.0	89.5	0.0	82.8	12.2	75.6	39.4	36.3	0.0	85.7
	3.7	7.4	6.5	2.2	3.5	7.0	10.3	6.9	7.3	4.9	6.1	18.2	0.0	14.3
LW	1.8	91.2	3.3	93.5	0.0	90.3	0.0	100.0	0.0	86.1	30.3	45.5	36.4	50.0
	5.2	1.8	1.6	1.6	3.2	6.5	0.0	0.0	11.1	2.8	3.0	21.2	13.6	0.0
JU									2.6	3.7	54.8	21.4	33.3	42.9
									13.2	10.5	2.4	21.4	0.0	23.8
ML							2.5	80.0			51.2	19.5	28.2	30.8
							5.0	12.5			4.9	24.4	23.1	17.9
CA							53.8	28.2	34.3	37.1			69.4	2.8
							2.6	15.4	2.9	25.7			2.8	25.0
PR									42.9	23.8	78.5	4.8		
									11.9	21.4	4.8	11.9		

4. Discussion

To prevent TBV infections in tulips, only high or absolute levels of resistance are of importance in a breeding programme. Therefore, in screening tests the observed ELISA value can be converted using a significant ELISA threshold value, which results in two classes (healthy and diseased plants). A high correlation was found between observed (average ELISA value) and converted (percentage healthy plants) ELISA values in the seedling experiments.

Distinct differences in the susceptibility could be detected between and within *Tulipa* populations in the seedling stage. The percentage of healthy plants was highly correlated when seedlings of the populations were one, three or five years old. This observation enables to select for resistance in the seedling stage; hence before the tulip plant reached maturity. Most of the resistant descendants were obtained using *T. fosteriana* 'Cantata' or 'Princeps, as one of the parents. Both cultivars showed absolute resistance in tests at clonal level (Eikelboom *et al.*, 1992).

The GCA effect explained a large proportion of the sum of squares in the diallel analysis. The combined other effects were of less importance. A model with only GCA effects described most of the variation. Furthermore the GCA value of each parent is associated with the level of resistance tested as clones (Eikelboom *et al.*, 1992). Backcrossings of the descendants will provide a more precise understanding of the inheritance.

A clear selection response was obtained by measuring the virus content in the leaves six weeks after infection of tulip seedlings. Although seedling selection more than doubled the percentage of resistant seedlings (24.9% = >63.3%) and the population size decreased (1568 = >441 seedlings), ± 27% of the selected plants were still susceptible.

This can be due to the fact that despite wounding the virus can not always be detected in the leaves with ELISA (Eikelboom *et al.*, 1992). A more sensitive virus detection technique, perhaps at DNA level, might be helpful. Furthermore, ± 28% of the plants with no flower breaking were normally discarded after seedling selection (missings). An explanation could be that the virus is not transported fast enough to the bulb before the leaf died (Romanow *et al.*, 1991). A clone test is necessary to find out if these missings are resistant or susceptible. Because the seedling test as described in this article is not so effective, laborious and therefore expensive optimizing this test or development of other tests need attention. A practical alternative is, to grow the seedlings some years outside surrounded with TBV infected tulips and without aphids control. Resistance of the seedlings can than be determined when the plants bloom. Plants remaining healthy have to be re-tested for their resistance in a clone test some years later. In the future, selection with molecular markers linked with TBV resistance genes might become feasible.

The seedlings inoculated in 1995 will be evaluated for infection by flower breaking in 1996. All seedlings with no flower breaking in 1995 or 1996 will be cultivated till 1998 and analyzed for resistance in a clone test. At that time, the exact percentage of resistant plants and missings can be determined. Since backcrossings of the resistant hybrids are necessary to obtain suitable cultivars for cut flower production, attention have to be paid to the possible F_1-sterility in the *T. gesneriana* x *T. fosteriana* (Darwin) hybrids.

References

Eikelboom, W., J.P. Van Eijk, D. Peters & J.M. Van Tuyl, 1992. Resistance to tulip breaking virus (TBV) in tulip. Acta Hort. 325: 631-636.

Hammond, J. & G.A. Chastagner, 1989. Field transmission of tulip breaking virus and serologically related potyviruses in tulip. Plant Disease 73: 331-336.

Romanow, L.R., J.P. Van Eijk & W. Eikelboom, 1986. Investigating resistance in tulip to tulip breaking virus and to its transmission. Acta Hort. 177: 235-240.

Romanow, L.R., J.P. Van Eijk, W. Eikelboom, A.R. Van Schadewijk & D. Peters, 1991. Determining levels of resistance to tulip breaking virus (TBV) in tulip (*Tulipa* L.) cultivars. Euphytica 51: 273-280.

Thomson, A., 1980. Infection trials with tulip mosaic virus. Acta Hort. 109: 469-471.

Van der Vlugt, C.I.M., 1994. Distribution and multiplication of iris severe mosaic potyvirus in bulbous *Iris* in relation to metabolic activity. PhD Thesis Agricultural University Wageningen, 130 pp.