

On the Hybrid F₁ Characteristics of Physiology, Biochemistry, Product Quality and Resistance to Black Shank in Oriental Tobacco

by

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SUMMARY

Experiments were conducted from 1996 to 1998 at the Hefei Institute of Economics and Technology and at the Oriental Experimental Station of the Zhejiang Province (China). Seven F₁ hybrids and three parental varieties of Oriental tobaccos were evaluated for the characteristics of photosynthetic and transpiration rates, esterase isozymes, resistance to black shank, quality and product potential from the 1996–1998 growing seasons.

Tobacco leaves had higher photosynthetic rates and many differences among genotypes in the early stage of plant vigorous growth compared with more mature leaves. However, transpiration rates were lower in the younger leaves and greater in the more mature leaves.

All the entries had four common bands (B1, B3, B4 and B6) of the esterase isoenzymes. Differences between entries resulted from in having or not having the B2 and B5 bands and color intensity differences of all the bands. These differences could be used to identify individual entries.

The F₁ hybrids Samsun × Toy and Samsun × Argjiro, compared with the CK Samsun control, had obvious heterotic vigor in the characteristics of product, for yield, quality and resistance to black shank. The F₁ hybrid Samsun × Toy maintained higher photosynthetic and transpiration rates in the two growth stages compared to other entries. However, the F₁ hybrid Samsun × Argjiro had higher photosynthetic rates and lower transpiration rates in the early growth stage and the two rates were lower in the later stage, but it maintained higher photosynthetic rates for the whole growth stage. Net photosynthetic rates had a significant positive corre-

lation with yield product, quality and resistance to black shank of the Oriental tobacco F₁ hybrids. [Beitr. Tabakforsch. Int. 19 (2000) 159–165]

ZUSAMMENFASSUNG

Die Untersuchungen wurden von 1996 bis 1998 in China am „Institute of Economics and Technology“ in Hefei und an der Forschungseinrichtung für Orienttabak in der Provinz Zhejiang (China) durchgeführt. Sieben F₁-Hybriden und drei Varietäten von Elterngenerationen des Orienttabaks der Anbauperiode 1996–1998 wurden hinsichtlich ihrer Eigenschaften bei Photosynthese- und Transpirationsrate, der Esterase-Isoenzyme, ihrer Resistenz gegenüber des Black Shanks, des Produktpotentials und der Qualität untersucht.

Im frühen Stadium des ausgeprägten Wachstums wiesen die Tabakblätter im Gegensatz zu vollentwickelten Blättern höhere Photosyntheseraten auf und es bestanden viele Unterschiede zwischen den Genotypen. Die Transpirationsraten hingegen waren in den jüngeren Blättern niedriger als in den älteren Blättern.

Alle Varietäten wiesen vier gemeinsame Banden (B1, B3, B4 und B6) der Esterase-Isoenzyme auf. Die Unterschiede zwischen den einzelnen Varietäten bestanden im Vorhandensein oder Nichtvorhandensein von B2 und B5 Banden und in Unterschieden bei der Farbintensität aller Banden. Diese Unterschiede konnten zur Identifizierung der Varietäten herangezogen werden.

Die F₁-Hybriden Samsun × Toy und Samsun × Argjiro hatten im Vergleich zur CK Samsun-Kontrolle eine deutliche heterotische Stärke bezüglich der Produkteigen-

schaften, des Ertrags, der Qualität und der Resistenz gegenüber des Black Shanks. Der F₁-Hybrid Samsun × Toy behielt im Vergleich zu anderen Varietäten in beiden Wachstumsstadien höhere Photosynthese- und Transpirationsraten. Der F₁-Hybrid Samsun × Argjiro hatte zwar im frühen Wachstumsstadium höhere Photosyntheseraten und niedrigere Transpirationsraten und im späteren Stadium waren beide Raten niedriger, die Photosyntheseraten blieben jedoch während des gesamten Wachstumsstadiums erhöht. Eine signifikante positive Korrelation der Nettphotosyntheseraten mit Ertrag, Qualität und Resistenz gegenüber Black Shank bei F₁-Hybriden des Orienttabaks konnte festgestellt werden. [Beitr. Tabakforsch. Int. 19 (2000) 159–165]

RESUME

Des essais ont été menés de 1996 à 1998 à l' "Institute of Economics and Technology" d'Hefei, et au Centre d'Expérimentation sur les tabacs d'Orient de la province de Zhejiang (Chine). Huit hybrides F₁ et trois variétés parentales de tabacs d'Orient ont été évaluées pour leurs caractéristiques en matière de photosynthèse et de transpiration, d'isoenzymes de l'estérase, la résistance au black shank, le potentiel produit et la qualité, lors des campagnes de culture 1996–1998.

Les feuilles de tabac présentaient des taux de photosynthèse et des différences inter-génotypes plus élevées au cours des premiers stades de développement que lors de phases plus tardives. Cependant, les taux de transpiration étaient plus faibles chez les jeunes feuilles et plus élevés chez les feuilles plus matures.

Toutes les variétés avaient quatre bandes (B1, B3, B4 et B6) d'isoenzymes de l'estérase en commun. Des différences entre variétés se sont caractérisées par la présence ou l'absence des bandes B2 et B5 et par des différences en intensité de la couleur de toutes les bandes, pouvant être utilisées pour identifier les variétés.

Les hybrides F₁ de Samsun × Toy et Samsun × Argjiro, comparés au CK Samsun témoin, avaient une vigueur hétérotis très nette pour les caractéristiques du produit, du rendement, de la qualité et de la résistance au black shank. L'hybride F₁ Samsun × Toy a maintenu des taux plus élevés de photosynthèse et de transpiration au cours des deux stades de croissance comparés aux autres variétés. Toutefois l'hybride F₁ Samsun × Argjiro avait un taux de photosynthèse plus élevé et un taux de transpiration plus faible au cours du premier stade de croissance et ces deux taux étaient plus bas au cours du deuxième stade, mais les taux de photosynthèse pour l'ensemble de la croissance demeuraient à des niveaux élevés.

Pour les hybrides F₁ de tabacs d'Orient une corrélation positive significative des taux nets de photosynthèse avec le rendement du produit, sa qualité et sa résistance au black shank a été observée. [Beitr. Tabakforsch. Int. 19 (2000) 159–165]

INTRODUCTION

In China, Oriental tobacco cultivars from abroad have been used for a long time and these old cultivars do not provide the quality product presently required. It is important to select new cultivars which meet the requirements of Oriental tobacco cultivation in China. Since general methods of cross-breeding need 8 to 10 years for one cycle, utilizing the F₁ hybrid is a better choice in many crops (2, 6) and studies on tobacco heterosis have been carried out for many years in flue-cured (3) and burley tobacco (7). However, utilizing heterosis in Oriental tobacco for theoretical studies and practical application is extremely limited. Therefore, a series of experiments were conducted to select F₁ hybrids of Oriental tobaccos with application to product potential and to study their characteristics of physiology, biochemistry and resistance to black shank of Oriental tobaccos.

MATERIALS AND METHODS

Oriental tobacco entries tested

Seven F₁ hybrids and three varieties of Oriental tobaccos, ms-Basma, ms-Basma × Argjiro, Samsun × Toy, T-Samsun × Basma, ms-Samsun, Samsun × Argjiro, Samsun × Basma, Toy × Samsun, Basma × Samsun and Samsun, were evaluated in the following experiments.

Experimental methods

Comparisons of yields and economic returns among various Oriental tobacco entries

The experiments were carried out at Xinchang County and Sheng County in the Zhejiang province from 1996 to 1998 in randomized blocks with three replications. Yields, economic return, natural resistance to black shank and agronomic characteristics were investigated. Leaves were sampled from each entry at each location each year for chemical analysis and smoke quality determination.

Photosynthetic and transpiration rate assays

A pot experiment, each cultivar with 8 pots, was conducted at the experimental station of the Hefei Institute of Economics & Technology. Photosynthetic and transpiration rates of all entries tested were measured on plants growing in pots, 8 pots per entry, with a leaf chamber analyzer (type 4) at the main growth stages (40 and 60 days after transplanting, respectively). At the first assay, 40 days after planting, the light level was $145.2 \pm 1 \mu\text{mol m}^{-2} \text{s}^{-1}$, temperature: $30.1 \pm 0.5^\circ\text{C}$, H₂O: $15.8 \pm 0.6 \text{ mbar}$, and CO₂: $406 \pm 3.6 \text{ ppm}$; at the second assay, 60 days after planting, the light level was $169 \pm 1 \mu\text{mol}$

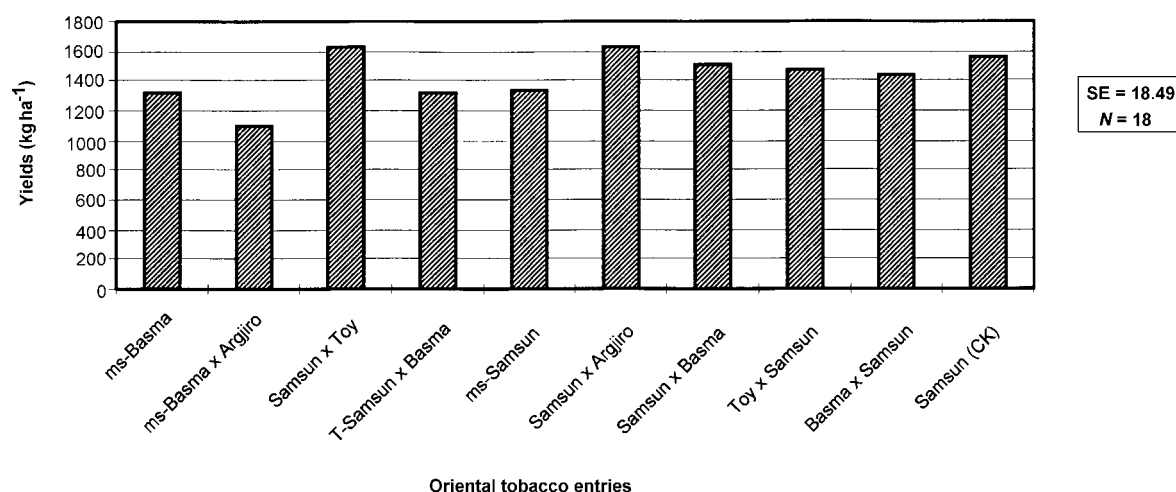


Figure 1.
Yields of ten Oriental tobacco entries

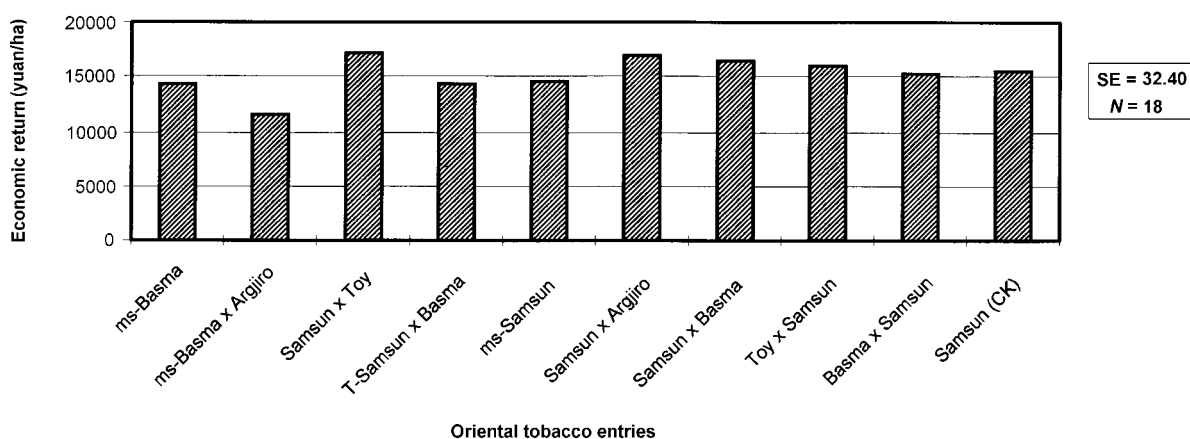


Figure 2.
Economic returns of ten oriental tobacco entries (¥1 [yuan] = US\$0.121)

$\text{m}^{-2} \text{ s}^{-1}$, temperature: 33.2 ± 0.5 °C, H_2O : 19.8 ± 0.7 mbar, and CO_2 : 385.4 ± 3.2 ppm.

Esterase isozymes assay for Oriental tobacco entries

The esterase of seedling leaves was assayed for all the tobacco entries tested at the Hefei Institute of Economics & Technology. The polyacrylamide gel electrophoresis system according to the method described by BAI (1) was used with slight modification. Bands of the isozymes were scanned with a Shimazu CS-9000 thin layer scanning chromatograph.

Testing of resistance to black shank of tobacco

The resistance to the pathogenicity of *P. parasitica* var. *nicotianae* was determined on the 10 Oriental tobacco entries in pot experiments (15 pots per entry) at the Hefei Institute of Economics & Technology. The

method of inoculation was to pour a zoospore suspension, 30 mL per pot with 5.0×10^4 zoospores $\cdot \text{mL}^{-1}$ into the soil around the plant roots, injecting the zoospore suspension into the stem and stuffing hyphae with approximately 2 mm^2 solid medium culturing the pathogenic fungi into the stem cuts. Determination of the disease severity was based on the symptom appearing early and late.

RESULTS

Yields and yield values of ten Oriental tobacco entries

Ten Oriental tobacco genotypes, 3 varieties and 7 hybrids, were tested in fields at two locations from 1996 to 1998 to compare their yields and economic returns. The data were analyzed with analysis of variance combined over locations and years for yield potentials and values.

Table 1.
Chemical compositions and smoking quality of ten Oriental tobacco entries

Genotypes	Nicotine (%)	Total N (%)	Reducing sugar (%)	Protein (%)	Smoking quality ^a
ms-Basma	1.58	2.91	12.16	16.47	m ⁺
ms-Basma × Argjiro	1.53	2.77	11.75	15.65	m
Samson × Toy	1.21	2.96	12.03	17.19	m ⁺
T-Samson × Basma	1.65	3.08	10.31	17.46	m
ms-Samson	1.24	2.84	12.71	16.40	m ⁻
Samson × Argjiro	1.16	3.02	12.14	17.62	m ⁺
Samson × Basma	1.31	3.03	11.63	17.52	m
Toy × Samson	0.86	2.57	13.55	15.13	m ⁺
Basma × Samson	1.22	2.89	11.06	16.74	m ⁺
Samson (CK)	0.79	3.10	11.68	18.52	m
ANOVA^b P-value	0.025	0.05	0.05	0.025	
SE^c	0.133	0.11	0.184	0.224	

^am = medium smoking quality level based on national standards

^bANOVA = analysis of variance

^cSE = standard error of the means of ten genotypes (*n* = 6)

The differences among genotypes were overall significant at the *P* = 0.05 level. The results were shown as follows: Oriental tobacco hybrids Samsun × Toy and Samsun × Argjiro had higher yields at 1627.5 kg ha⁻¹ than the currently planted cultivar Samsun at 1563.0 kg ha⁻¹ (Figure 1). These differences are significant at the 0.05 level with the Duncan's multiple range test. The hybrids Samsun × Toy and Samsun × Basma, compared respectively with their reciprocal hybrids Toy × Samsun and Basma × Samsun had higher yields, which were significantly different at the 0.05 and 0.01 probability level, respectively. All the hybrids with higher yields also had higher output values compared with the CK Samsun control (Figure 2). The same pattern was measured for Samsun × Toy and Samsun × Basma hybrids in that the hybrids with the highest yield had higher economic returns than the reciprocal.

Chemical compositions and smoking qualities of Oriental entries

Nicotine, total nitrogen, reducing sugar and protein are important characteristics of Oriental tobacco cured-leaves. There were significant differences among the entries for all the characteristics tested except for the smoking quality (Table 1). SE and *n*, Duncan's multiple range parameters, are shown in the footnote.

The mean content of nicotine was 1.26% and the coefficient of variance (CV) was 0.23. T-Samsun × Basma had the highest nicotine content of all the entries (1.65%) and contained twice the nicotine content of the Samsun control which had the lowest nicotine content of all the entries tested (0.79%). Total nitrogen, reducing sugar and protein characteristics varied among the materials tested

with CVs of 2.92%, 11.90% and 16.87%, respectively. However, the reciprocal cross hybrids of Samsun with Toy and Samsun with Basma, had significant differences for all the four chemical characteristics except for the reducing sugar in the Samsun × Toy hybrids.

Smoking quality was assessed by organoleptic test and its grades were determined based on national standards. There were only slight differences among the entries and all of them were acceptable.

Photosynthetic and transpiration rates of various Oriental tobaccos

Leaf chamber analyzer-4 was used to measure photosynthesis and transpiration characteristics in the main plant growth stages.

Fourty days after transplanting there were larger differences in photosynthetic rates among the entries (Figure 3). The highest photosynthetic rate with 5.69 μmol m⁻² s⁻¹ of ms-Basma was 10 times greater than that of the lowest, with 0.54 μmol m⁻² s⁻¹ of Basma × Samsun. Hybrids in which Samsun was used as female parent had significant higher photosynthetic rates than their reciprocal hybrids.

Sixty days after transplanting the differences in photosynthetic rates were smaller than 40 days after transplanting (Figure 3). Not only did the range become smaller (1.08–4.13 μmol m⁻² s⁻¹), the mean photosynthetic rate also was lower.

However, the hybrid Samsun × Toy had a higher photosynthetic rate 40 days after transplanting than the reciprocal cross hybrid Toy × Samsun. There was only a slight difference between these two hybrids 60 days after transplanting when the two hybrids appeared higher

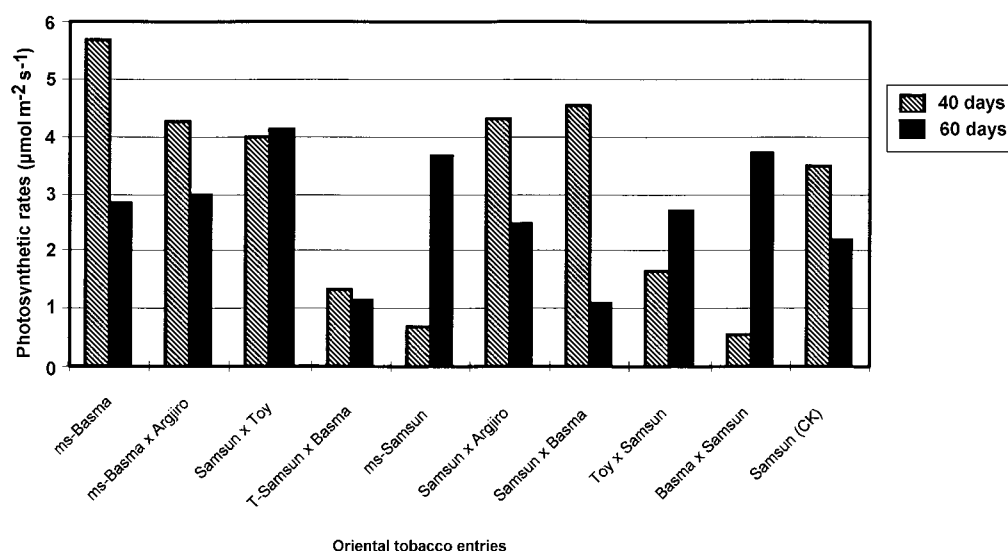


Figure 3.
Photosynthetic rates of Oriental tobacco entries in the two growth stages

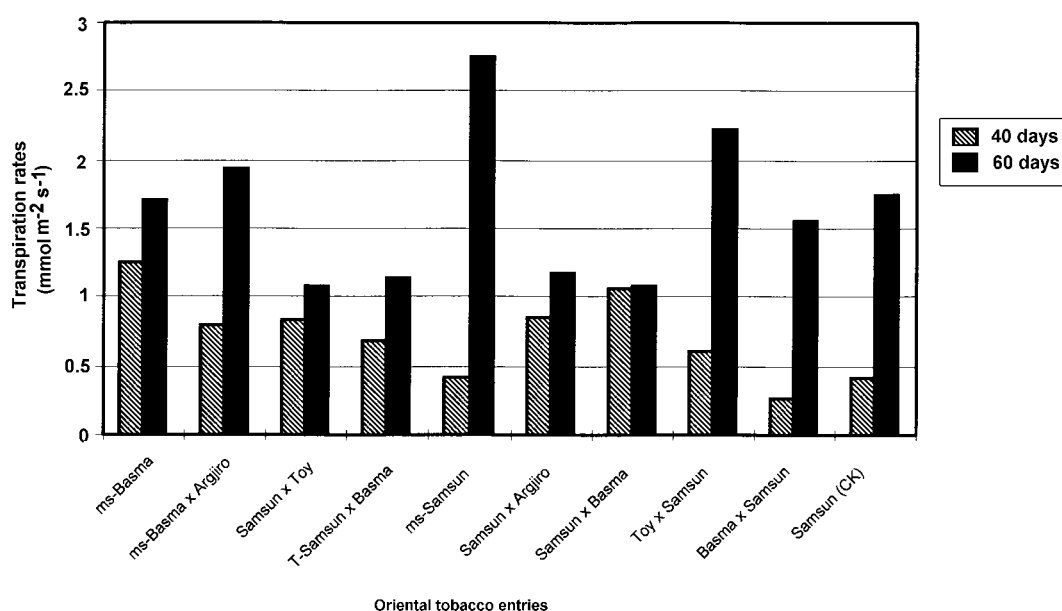


Figure 4.
Transpiration rates of Oriental tobacco entries in the two growth stages

photosynthetic rates. The hybrid Samsun × Basma with its reciprocal cross hybrid Basma × Samsun had significant differences at both growth stages tested. Forty days after transplanting the photosynthetic rate of Samsun × Basma was significantly higher than its reciprocal cross hybrid Basma × Samsun, however, the opposite result was measured 60 days after transplanting.

There were less differences in the transpiration rate among the entries 40 days after transplanting than 60 days after transplanting (Figure 4). In the early growth stage the range of transpiration rates ranged from 0.26–1.26 mmol and the mean transpiration rate of the 10 entries was 0.719 mmol. The differences between

reciprocal cross hybrids of Samsun and Basma were significant but the differences between the Samsun and Toy reciprocal hybrids were not significantly different. In these reciprocal hybrids the hybrids with Samsun as female parent had a higher transpiration rate.

Sixty days after transplanting the plants had remained higher in their transpiration rates with a mean of 1.64 mmol and a range from 1.08 to 2.76 mmol. The two reciprocal cross hybrids of Samsun and Basma and Samsun and Toy with their reciprocal hybrids, showed contrary results compared to 40 days after transplanting in that hybrids with Samsun as the female parent had remained lower in their transpiration rates.

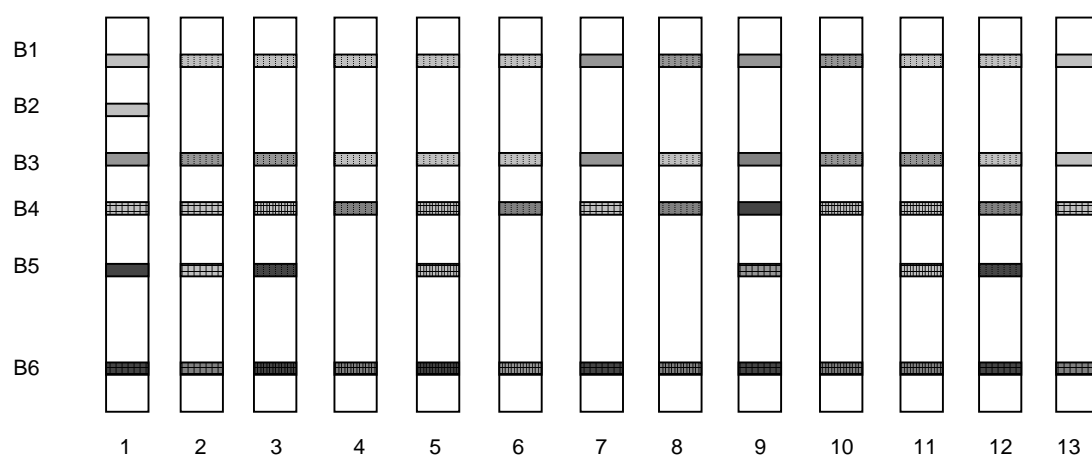


Figure 5.
Band models of esterase isozymes of Oriental tobacco entries

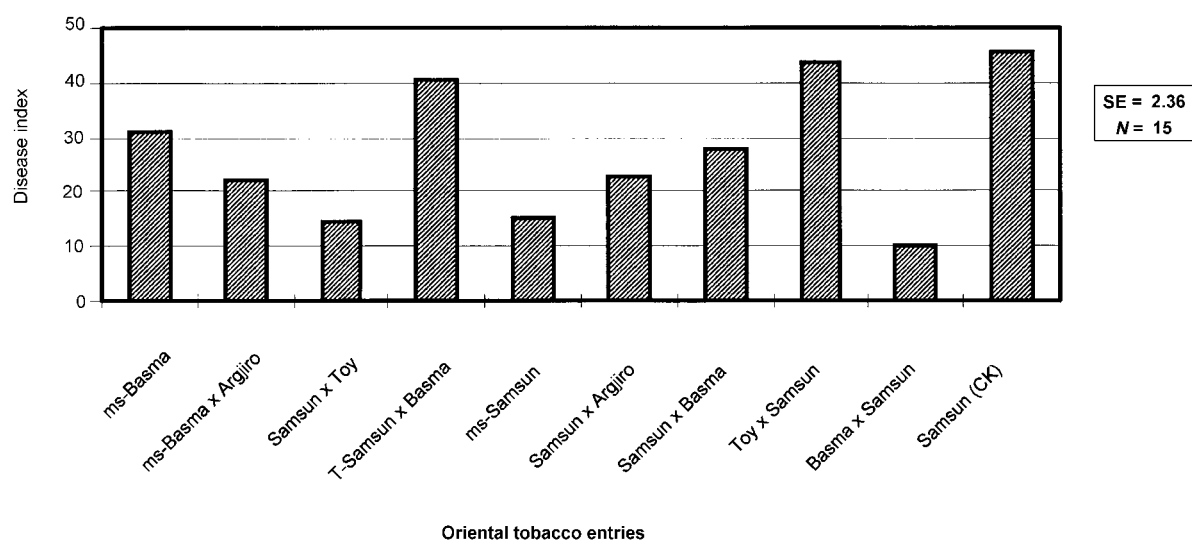


Figure 6.
Resistance to black shank

There were marked differences in the transpiration rate between the two growth stages for the same genotypes (Figure 4). The largest difference in the transpiration rate between the two growth stages was in ms-Samsun and the smallest difference in Samsun × Basma.

Esterase of Oriental tobacco entries

On the results of scanning the gels with a Shimazu CS-9000 thin layer scanning chromatograph the modal figures are shown in Figure 5.

The Oriental tobacco genotypes tested had many common bands of esterase isozymes and had some differences (Figure 5) among the genotypes, which was similar to the results obtained by CHEN *et al.* (3). There were four

common bands, B1, B3, B4 and B6, which illustrate genetic stability of Oriental tobacco genotypes. The B2 band was found only in the hybrid T-Samsun × Basma and only half of the genotypes had the B5 band. In addition, the same bands of the isozyme had color degree differences between the entries tested and these differences could be used to identify Oriental varieties (6).

Resistance to black shank disease

Tobacco black shank (*Phytophthora parasitica* var. *nicotianae*) is an important root and stem disease spread by soil transmission which affects the production of Oriental tobacco in China. Assessment of Oriental tobaccos for resistance to black shank was conducted with two

methods, pouring zoospore suspension into the soil around the plant roots and stuffing hyphae into the stem cuts. The mean disease index of the plants tested with the two methods is shown in Figure 6. The differences in the inoculation methods on resistance of plant to the disease have been discussed in another manuscript (4).

The hybrids Samsun \times Toy and Basma \times Samsun showed high resistance to the disease. Toy \times Samsun, T-Samsun \times Basma and Samsun (CK) were susceptible and had a high disease index. It is interesting to note that in the two reciprocal cross hybrids where Samsun was the female parent, Samsun \times Basma was susceptible and Samsun \times Toy was resistant.

DISCUSSION

Cytoplasm effect on the Oriental hybrids

Differences between reciprocal cross hybrids were found in yields, economic returns, chemical composition, photosynthetic and transpiration rates, esterase bands of isozymes and resistance to black shank. When Samsun was used as the female parent significant positive effects for most characteristics were measured. This result might be due to its adaptability to local conditions since it has been cultivated and selected in China for a long time. This result suggests that choosing parents for the adaptability of the female parent may be significant.

Usability of Oriental tobacco hybrids

Burley tobacco F_1 hybrids have been planted for a long time, but flue-cured tobacco hybrids have not shown superior performance and have not been used extensively for better usability in production. This investigation has shown that Oriental tobacco F_1 hybrids have potential usability in field cultivation, especially such as Samsun \times Toy and Samsun \times Argjiro, which had obvious heterotic vigor for the characteristics of yield, quality and resistance to disease compared with Samsun (CK).

Identification of Oriental tobacco with isozymes

Isozymes have been used to identify varieties in many crops (2, 3, 7) but identification of Oriental tobacco hybrids with isozymes has not been reported. There were differences in numbers and color changes of the esterase isozymes between entries, which had some stability and these might be used to identify Oriental tobacco hybrids.

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