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Evaluation of Tobacco Cultivars for Resistance to Races of *Phytophthora nicotianae* in South Africa

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Abstract

Black shank caused by *Phytophthora nicotianae* is a destructive root and stem disease of cultivated tobacco. *P. nicotianae* isolates associated with black shank in South Africa were selected in order to evaluate their aggressiveness, designate races of *P. nicotianae* and to determine which tobacco cultivars are resistant to them. Stem inoculations were conducted in the greenhouse using 32 *P. nicotianae* isolates from different tobacco growing regions in South Africa. The 32 *P. nicotianae* isolates differed significantly in levels of aggressiveness. Eight isolates were selected on the basis of geographical origin and virulence for race characterization using a set of differential tobacco cultivars. South African race 0 and race 1 isolates were used to evaluate black shank resistance of 11 commercially planted tobacco cultivars. Race 0 and 1 of *P. nicotianae* occurred in most of the tobacco growing regions. Commercially planted cultivars differed significantly in their resistance to race 0 and 1. Cultivars Vuma/3/46 and LK3/46 were highly resistant to both race 0 and 1 while cultivars LK33/60 and OD1 were highly resistant to race 0 but susceptible to race 1. Results of this study provide valuable information on cultivar resistance and selection of *P. nicotianae* isolates for future breeding programs in South Africa.

Introduction

Black shank, caused by *Phytophthora nicotianae*, is the most costly disease of tobacco in the United States (Bickers, 1992; Melton, 1998) and South Africa (Prinsloo, 1994; Van Jaarsveld, 1995). Plants infected early in the season are severely stunted and collapse before leaves are sufficiently mature to be harvested (Csinos and Bertrand, 1994). Disease symptoms vary with age of plants and include wilting, leaf yellowing and tissue degeneration inside the base of blackened stems (Lucas, 1975; Shew, 1991).

In South Africa, tobacco is cultivated in three main areas. These include Mpumalanga (Lowveld Golden Leaf Ltd. in the Lowveld trading area), North-West Province (MKTV Co-operative in the MKTV trading area) and Northern Province (Potgietersrus Co-operative in the PTK trading area). Approximately 25 million kg flue-cured and 4.8 million kg air-cured tobacco is produced annually in these regions (Anonymous, 1998). The gross income from agricultural production of tobacco in South Africa amounts to 423 million Rand (J. S. Venter, Tobacco Board RSA, PO Box 26100, Arcardia, 0007, pers. comm.). However, the occurrence of black shank in most of the South African tobacco growing regions poses a serious threat to this industry (Prinsloo, 1994; Van Jaarsveld, 1995).

Four different *P. nicotianae* races (race 0, 1, 2 and 3) have been reported to occur on tobacco. These races are defined by their ability to infect various cultivars with different resistance genes (Apple, 1957; Apple, 1962; Prinsloo and Pauer, 1974; McIntyre and Taylor, 1978). Apple (1962, 1967) first described a *P. nicotianae* strain that was non-pathogenic to *Nicotianae plumbaginifolia* Viv. as race 0 and a highly virulent strain as race 1. *P. nicotianae* race 2 is defined by the differential response of 3 cultivars, Kentucky 14 × L8, Burley 21 × L8, and Delcrest 202. Delcrest 202 has a single dominant gene resistant to race 2 (Lamprecht, 1973; Lamprecht et al., 1974; Prinsloo and Pauer, 1974).

P. nicotianae race 3 is able to overcome resistance in cigar wrapper tobacco and is cold tolerant (Taylor, 1975; McIntyre and Taylor, 1978). It has been suggested that cold tolerance and ketose production are associated with virulence, however, there is no firm genetic data to support this hypothesis (Taylor, 1975; McIntyre and Hankin, 1977).

The continuous large-scale cultivation of tobacco cultivars with resistance to a single race of *P. nicotianae* is not desirable as it increases the selection of new virulent races. For example, race 1 *P. nicotianae* isolates were found after continuous cultivation of tobacco cultivars with resistance to race 0 of *P. nicotianae* in North Carolina (Apple, 1967) and Georgia USA (Csinos and Bertrand, 1994). In South Africa, *P. nicotianae* race 1 appeared in the MKTV trading area after only two years of growing the cultivar Delcrest 202, which has resistance to race 2 (Lamprecht, 1973; Lamprecht et al., 1974; Prinsloo and Pauer, 1974). The occurrence of *P. nicotianae* race 1 has increased to such an extent in the MKTV area, that Prinsloo (1994) speculated that race 2 is no longer present or occurs at undetectable levels in the soil.

Integrated control programs for black shank include crop rotation, fungicide application and planting resistant cultivars (Melton, 1998). Planting tobacco cultivars with high levels of black shank resistance represents the most economical long-term solution for growers. Five main sources of black shank resistance have been identified in worldwide tobacco breeding programs. They include the cigar tobacco cultivars Florida 301 (Tisdale, 1931; Wernsman et al., 1974) and Beinhart 1000–1 (*Nicotianae tabacum*) (Silber and Heggestad, 1963; Chaplin, 1966), the flue-cured cultivar Coker 371-Gold (Carlson et al., 1997), *Nicotianae longiflora* (Valleau et al., 1960) and *Nicotianae plumbaginifolia* (Chaplin, 1962).

South African breeding programs for tobacco cultivars with *P. nicotianae* resistance have been only partially successful (Van Jaarsveld, 1995). Growers have reported an increase in black shank, suggesting the appearance of new races or the emergence of more aggressive *P. nicotianae* populations (Van Jaarsveld, 1995). The objectives of this study were therefore to assess the variation in aggressiveness in the South African *P. nicotianae* population, to characterize isolates of the pathogen in terms of race and to assess the resistance to *P. nicotianae* races 0 and 1, in tobacco cultivars commercially deployed in South Africa.

Materials and Methods

Isolates

P. nicotianae isolates were collected from commercial fields planted with TL33, LK33/60, LK30/40/60, OD1, OD272 and CDL28. Thirty-eight soil samples were collected at the roots of plants with black shank symptoms. Soil samples were collected at 2 metre intervals in tobacco fields that are known for the occurrence of severe black shank. Soil samples were baited up to three times using citrus leaf discs (Grimm and Alexander, 1973). We also randomly collected 38 infected plants for direct isolation. Infected tissue was surface sterilized in 70% ethanol and rinsed with sterile water. This was followed by the excision and transfer of 6 mm square pieces of infected tissue to selective Pimaricin, Ampicillin, Rifampicin, PCNB, Hymexosal (PARPH) media (Tsao and Guy, 1977).

After incubation at 26 °C for 2–3 days, fungi growing from citrus discs and tobacco tissue were examined. Isolates identified as *P. nicotianae* were subcultured onto new plates containing selective media (PARPH) until cultures were clean. These *P. nicotianae* isolates were then transferred to potato dextrose agar (Biolab, Midrand) for aggressiveness studies. All the isolates were identified by morphological characterization of colony characteristics and microscopic examination, especially for the presence of papillate sporangia.

The 33 isolates of *P. nicotianae* acquired during this study were obtained as follows: 21 isolates from four farms in the Lowveld area, six isolates from two farms in the MKTV area and four isolates from one farm in the PTK area. We also included two isolates, each representing *P. nicotianae* races 2 and 0 as positive controls. These *P. nicotianae* isolates were identified as race 0 (Masuka and Namichila, 1996) and 2 (Prinsloo and Pauer, 1974) based on stem and soil inoculation pathogenicity studies on tobacco cultivars with different resistant genes. The race 0 isolate, P25 (CMW 6917), was obtained from the Kutsaga Tobacco Research Board, Harare, Zimbabwe, whereas the race 2 isolate, CMW 6914, was supplied by the ARC-Institute for Industrial Crops, Rustenburg, South Africa. All isolates are maintained in the culture collections of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and the Cooperative Research Center for Tropical Plant Pathology, University of Queensland, Australia.

Variation in aggressiveness

Two cultivars, TL33 and Hicks, were used to assess variation in aggressiveness of the *P. nicotianae* isolates. These cultivars are moderately resistant and susceptible, respectively, to black shank (Jones and Mann, 1958; Lamprecht and Prinsloo, 1977).

The *P. nicotianae* isolates used in aggressiveness tests were incubated on potato dextrose agar (Biolab, Midrand) at 27 °C in the dark for 5 days. Agar disks (5 mm in diameter), taken from the actively growing edges of these cultures were used to inoculate four-week-old plants. The cambium of the test plants was removed with a cork-borer (5 mm diam.), 2 cm above ground level. A disc of agar, colonized with *P. nicotianae*, was placed into the wound and sealed with parafilm. The two cultivars were inoculated with each of the 32 *P. nicotianae* isolates. The experimental design was a randomized complete block design with 8 replicates of 1 plant each. An equal number of control plants were inoculated with sterile disks of agar. The entire trial was repeated once. In each case the plants

were examined for lesion lengths after nine days. Re-isolations were made from these lesions on a medium containing Pimaricin (Tsao and Ocano, 1969) to verify that the inoculated pathogen was directly responsible for the lesion that developed. Data were statistically analyzed for variance and differences among isolates. Means were tested for significance by Tukey's procedure. The software used was developed by Dr A.J. Louw (AgDev-Gabon S.A., PO Box 206, Tzaneen, 0850, RSA) and based on principles presented by Steel and Torrie (1980).

Race designation

Eight isolates were used for the designation of races. Isolates were selected based on their geographical distribution and relative aggressiveness. These isolates included four isolates from the Lowveld area, two from the MKTV area, one from the PTK area and one from Zimbabwe (CMW 6917). A set of seven differential tobacco cultivars that are generally used for race identification (Nielsen, 1995) (Table 1), were inoculated with each of the selected eight *P. nicotianae* isolates as described above. In this test, eight-week-old tobacco plants were used and the inoculations, measurements and statistical analyses were performed as described above. Plants were examined for lesion length after seven days.

Evaluation of resistance in commercial cultivars

Three isolates of *P. nicotianae* representing race 0 and three isolates representing race 1 from South Africa were used to evaluate resistance to *P. nicotianae* in 11 commercially used cultivars. Ten flue-cured commercial cultivars were selected from the Lowveld area (LK3/46, LK30/40/60, LK33/60 and Vuma/3/46), the MKTV area (OD1, OD272), Zimbabwe (T20) and North Carolina (Speight G-108, K149 and MDH). A single air-cured cultivar CDL28 from the PTK area was also included. Eight-week-old plants were inoculated with each of the six test isolates. The inoculations, measurements and statistical analyses were performed as described above.

Results

Isolates

P. nicotianae was isolated from all the farms surveyed in this study (Table 2). All these isolates were characterized by the presence of papillate sporangia. Only 25 *P. nicotianae* isolates were recovered from the 38 soil samples tested. The reason for this relatively unsuccessful baiting is probably due to applications of fungicides that reduce successful baiting of *P. nicotianae* from soil. Likewise, the use of fungicides on plants may have limited the relative success of isolations from plant tissue (Van Jaarsveld et al., 2002). Only eight *P. nicotianae* isolates were recovered from infected plants.

Variation in aggressiveness

Significant differences ($P < 0.05$) in lesion length were associated with inoculations with different *P. nicotianae* isolates on Hicks and TL33 (Table 3). Variation in aggressiveness, as reflected by lesion length, was greater on Hicks (6.6 mm to 137.3 mm) than on TL33

Table 1
Tobacco cultivars used for *P. nicotianae* race identification worldwide

Cultivar	Level of resistance to *P. nicotianae*		References
	Race 0	Race 1	
Hicks	None	None	Csinos (1999)
KY 14	None	None	Nielsen (1995)
KY 14 × L8	High	None	Nielsen (1995)
Coker 371–Gold	High	None	Carlson et al. (1997)
Burley 37	Moderate	Moderate	Collins et al. (1971)
NC 2326	Low to moderate	Low to moderate	Wernsman et al. (1974), Csinos (1999)
Beinhart 1000–1	Very high	Very high	Chaplin (1966), Silber and Heggestad (1963)

Table 2
Origin of *Phytophthora nicotianae* isolates collected in this study

Area	Site	Farm	Number of soil samples	Isolates from soil	Isolates from plants	Total number of isolates
Lowveld	Karino	Barberton Boere	8	5	0	5
		Friedenheim	14	11	0	11
	Barberton	Dave Cooper	6	4	0	4
	Schagen	LTGA farm	4	1	0	1
MKTV	Rustenburg	Roelf Otterman	3	1	3	4
		Research Institute for Tobacco & Cotton	0	0	2	2
PTK	Potgietersrus	De Wet Roos	3	2	2	4
Zimbabwe		Kutsaga	0	0	1	1
Total			38	25	8	33

Table 3
Analysis of variance for lesion lengths on two tobacco cultivars (TL33 and Hicks) inoculated with 32 *P. nicotianae* isolates[a]

Source of variation	d.f.	S.S.[b]	*F*-value	Significance[c]
Treatments[d]	63	8837	12.5	**
Cultivars	1	214943	304.0	**
Isolates	31	8138	11.5	**
Cultivars × Isolates	31	2888	4.1	**
Error	448	707	–	–

[a] Each *P. nicotianae* isolate was inoculated into eight plants of each cultivar;
[b] Sum of squares;
[c] Significance indicated by ** at P = 0.01 according to Tukey;
[d] Treatments consist of all possible combination of 32 *P. nicotianae* isolates and 2 cultivars.

(7.5 mm to 64.0 mm). The average lesion length on Hicks (75.1 mm) was significantly higher (Tukey's LSD cultivars 0.05 = 11.55) than on TL33 (36.9 mm) (Table 3).

A corresponding relationship in aggressiveness of *P. nicotianae* isolates was observed on the cultivars Hicks and TL33. There were exceptions however, since the *P. nicotianae* isolate (CMW 6914; race 2) was characterized as moderately pathogenic on Hicks, but not very pathogenic on TL33. This is likely to be the presence of *P. nicotianae* race 2-resistance genes in TL33 (Lamprecht et al., 1974; Lamprecht and Prinsloo, 1977). Another exception was *P. nicotianae* isolate CMW 6926, that is highly pathogenic on Hicks and caused the plants to die through girdling, prior to the development of typical elongated lesions, which were characteristic of the other inoculations. This isolate showed the greatest lesion length on TL33. Furthermore, *P. nicotianae* was consistently re-isolated from inoculated plants.

Race designation

Significant differences ($P < 0.05$) were found in lesion lengths associated with various *P. nicotianae* isolates on the differential tobacco cultivars (Table 4). South African *P. nicotianae* isolates were identified as race 0 and race 1 by their differential response of KY14xL8 and Coker371–Gold. Both cultivars are resistant to race 0 and susceptible to race 1 of *P. nicotianae*. The Zimbabwe isolate, CMW 6917 (race 0) had average lesion lengths of 49.0 mm on KY14xL8 and 41.6 mm on Coker 371–Gold. The average lesion lengths for isolates CMW 6921, CMW 6916 and CMW 6922 on Coker 371–Gold and KY14xL8 did not differ significantly from those of the Zimbabwe isolate CMW 6917 and were thus identified as race 0 (Table 4). Average lesion lengths for the isolates CMW 6923, CMW 6924, CMW 6925 and CMW 6926 were, however, significantly greater (LSD 0.05 = 26.35) on Coker 371–Gold and KY14xL8 than race 0 isolates and were thus identified as race 1. Race 0 was found in all areas while race 1 was only identified in MKTV and the Lowveld area.

Evaluation of commercial cultivars

Significant differences ($P < 0.05$) in lesion length were associated with inoculations of race 0 and 1 *P. nicotianae* isolates on various commercial tobacco cultivars (Table 5). Average lesion length associated with race 1 isolates was significantly greater on OD1 (81.0 mm), MDH (70.9 mm) and LK33/60 (99.0 mm) than with race 0 isolates. Inoculations with race 0 isolates resulted in small lesions on OD1 (50.5 mm), MDH (53.7 mm) and LK33/60 (57.9 mm) reflecting the resistance of these cultivars to race 0. Average lesion length associated with race 0 isolates ranged from 50.5 mm (OD1) to 116.5 mm (LK30/40/60). For race 1 isolates, average lesion lengths ranged from 37.7 mm (LK3/46) to 151.7 mm (CDL28). No significant differences ($P < 0.05$) were found in lesion length associated with race 0 and 1 isolates on the commercial cultivars Vuma/3/46, T20, OD272, K149 and LK30/40/60.

Discussion

In this study, South African isolates of *P. nicotianae* exhibited significant ($P < 0.05$) differences in aggressiveness to *N. tabacum* cultivars. Yet this is not

Table 4
Lesion lengths (mm) after inoculation with eight *P. nicotianae* isolates on seven differential tobacco cultivars

			Tobacco cultivars[a]						
Isolates	Origin	Race	KY14	Hicks	NC 2326	Burley 37	KY14xL8	Coker 371–Gold	Beinhart 1000–1
CMW 6917	Zimbabwe	0	97.37 [b] a	48.8 a	43.6 a	46.5 a	49.0 a	41.6 ab	49.6 a
CMW 6922	Lowveld	0	120.3 ab[c]	79.0 b	56.8 ab	46.8 a	64.1 a–d	46.0 a–c	41.5 a
CMW 6921	PTK	0	123.1 a–c	78.3 b	53.5 ab	55.3 a	63.4 a–c	50.1 a–d	36.3 a
CMW 6916	MKTV	0	126.1 b–d	81.0 b	61.5 ab	48.4 a	49.8 ab	40.5 a	39.0 a
CMW 6926	Lowveld	1	128.0 b–e	85.5 b	72.3 b	54.4 a	87.4 c–e	68.8 c–e	45.3 a
CMW 6925	Lowveld	1	147.4 c-f	75.1 b	67.3 ab	55.9 a	119.0 f	82.6 ef	28.1 a
CMW 6924	Lowveld	1	181.5 g	90.8 b	67.6 ab	56.4 a	97.0 ef	96.3 f	33.3 a
CMW 6923	MKTV	1	189.5 g	94.8 b	63.3 ab	58.0 a	96.8 ef	71.6 c–f	45.8 a

[a] KY14 and Hicks are susceptible to *P. nicotianae*, while NC 2326 and Burley 37 have low to moderate resistance. KY14xL8 and Coker 371–Gold are resistance to race 0 and susceptible to race 1. Beinhart 1000–1 has the highest resistance to race 0 and 1;
[b] Each value represents the mean of 8 replicates;
[c] Within each column, mean values followed by the same letter are not significantly different according to Tukey's LSD (0.05) = 26.35.

Table 5
Lesion lengths on 11 commercial tobacco cultivars after inoculation with isolates of *P. nicotianae* race 0 and 1[a]

	P. nicotianae isolates							
	Race 0				Race 1			
Cultivar	CMW 6922	CMW 6921	CMW 6916	AV[b]	CMW 6925	CMV6924	CMW 6923	AV
OD1	42.4	54.0	55.3	50.5 ab[c]	64.3	72.3	106.6	81.0 g–l
MDH	58.3	51.9	50.9	53.7 bc	65.1	70.9	76.6	70.9 d–j
LK33/60	51.1	64.9	57.8	57.9 b–e	77.0	105.6	114.5	99.0 m–q
Vuma/3/46	59.9	58.8	62.9	60.5 b–f	58.8	71.3	73.1	67.7 d–g
T20	75.5	55.1	79.8	70.1 d–h	56.8	60.0	56.3	57.7 b–d
LK3/46	78.5	43.9	88.4	70.2 d–i	47.6	33.5	32.0	37.7 a
Speight G–108	62.6	77.0	76.0	71.9 d–k	125.1	90.8	122.3	112.7 p–t
OD272	75.8	82.3	95.5	84.5 h–m	97.3	92.8	79.4	89.8 l–o
K149	111.3	83.5	100.6	98.5 m–p	90.3	84.3	80.9	85.1 h–n
CDL28	91.1	92.1	125.0	102.8 o–r	172.7	158.0	124.4	151.7 u
LK30/40/60	113.6	126.5	109.4	116.5 r–t	110.9	110.0	92.7	104.5 o–s

[a] Each value represents the mean of 8 replicates;
[b] Average lesion lengths of 3 *P. nicotianae* isolates;
[c] Within each column, mean values followed by the same letter are not significantly different according to Tukey's LSD (0.05) = 15.3.

unusual, since similar results have also been reported elsewhere (Lucas, 1975; Colas et al., 1998). Colas et al. (1998) for example, showed that within a *P. nicotianae* population from tobacco hosts, large variation in aggressiveness exists between isolates.

Variation in aggressiveness to tobacco plants may lead to a shift in South African *P. nicotianae* populations. This would be similar to the replacement of one genotype of *Phytophthora infestans* by another more aggressive genotype as described in the potato fields in the Columbia Basin, USA (Miller et al., 1998). For example, during a tobacco season, the more aggressive isolates of *P. nicotianae* from MKTV and Lowveld areas are likely to colonize plant tissue faster. These aggressive isolates might also sporulate faster, thereby increasing their chance of dispersal and dissemination. Aggressive isolates also have the competitive advantage of colonizing a greater number of plants. Plants infected by aggressive isolates may therefore have severe black shank symptoms early in the tobacco-growing season. Consequently, fewer tobacco leaves of poor quality would be harvested before plant death.

In this study, we have shown that both races 0 and 1 of *P. nicotianae* occur in South Africa. This is consistent with previous reports of race 0 in the Lowveld and race 1 in the MKTV area (Prinsloo, 1994; Nielsen, 1995). Yet our results represent the first report of the occurrence of race 0 in the MKTV and PTK area and race 1 in the Lowveld area. The occurrence of races 0 and 1 in these areas is a cause for great concern, since many tobacco cultivars planted here are resistant to only a single race of *P. nicotianae*. Cultivation of resistant cultivars is likely to result in the emergence of new races of *P. nicotianae* (Apple, 1957; Delon, 1991). Resistance may be overcome by only small changes in existing races due to a mutation in virulence alleles in the *P. nicotianae* population in a similar fashion as reported for the cloned and sequenced virulence allele's of *Cladosporium fulvum* (Joosten et al., 1994). This is consistent with the observation that new races were able to overcome resistance genes in introduced cultivars in Georgia, North Carolina, East Tennessee, and Australia (Gupton, 1972; Csinos and Bertrand, 1994; Robin and Guest, 1994; Melton, 1998).

Significant differences ($P < 0.05$) in resistance or tolerance to both races of *P. nicotianae* were discovered among the 11 different commercial tobacco cultivars analyzed in this investigation. Speight G-108, MDH and K149 had moderate resistance to races 0 and 1 as previously reported (Melton, 1993). The extensively planted air-cured cultivar CDL28 was found to be highly susceptible to the pathogen races 0 and 1 whereas the flue-cured cultivars Vuma/3/46 and LK3/46 were highly resistant to both races 0 and 1. LK33/60 and OD1 were highly resistant to race 0 but susceptible to race 1. This is in agreement with resistance ratings obtained for cultivars CDL28, Vuma/3/46, LK3/46 and LK33/60 with field trials (Scholtz, 1999) and pathogenicity studies where seedlings were inoculated on the roots with *P. nicotianae* race 0 isolates (Van Jaarsveld, 1995).

LK30/40/60 and T20 were rated as susceptible to moderately resistant, using the stem inoculation technique in this study. However, previous studies reported that these cultivars had high field resistance to *P. nicotianae* (Nielsen, 1995; Scholtz, 1999). Csinos (1999) proposed that there are at least two types of resistance acting against *P. nicotianae*: root resistance and stem resistance (Hendrix and Apple, 1967). Cultivars, such as T20 and LK30/40/60 have Florida 301-derived resistance that reduces root infection and slows epidemic development (Shew, 1983; Ferrin and Mitchell, 1986; Shew, 1987). Cultivars with Florida 301-derived resistance therefore have low stem resistance to *P. nicotianae* and thus explains the disease response of T20 and LK30/40/60 (Csinos, 1999).

We have shown that both races 0 and 1 occur in tobacco producing regions, where these races have not

been previously found. The practice of distributing tobacco seedlings from one geographical area to another after severe hail damage may play an important role in the distribution of *P. nicotianae* races in South Africa. Seedlings should be carefully examined for infection by *P. nicotianae* since infected plants have been the major agents of black shank spread in the USA and Australia (Tisdale and Kelley, 1926; Lucas, 1975; O'Brien and Davis, 1981). It is probable or even likely that the occurrence of new races in the Lowveld and MKTV area could be attributed to distribution via seedlings.

Results of this study have provided valuable information on selection of *P. nicotianae* isolates that might be used to augment for future breeding programs in South Africa. Knowledge on the occurrence and distribution of *P. nicotianae* races will assist breeders in the development and deployment of new cultivars. Furthermore, information from this study may be useful in advising farmers on appropriate cultivars to plant in various areas.

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