African Crop Science Journal, Vol. 13. No. 3, pp. 185-192, 2005
Printed in Uganda.

ISSN 1021-9730/2005 $ 4.00

RESPONSE OF CABBAGE CULTIVARS TO BLACK ROT INFECTION

H. SHIMELIS[1,2]
[1]Alemaya University, Department of Plant Sciences, P.O. Box 138, Dire Dawa, Ethiopia
[2]Present address: University of Limpopo, School of Agricultural and Environmental Sciences, Private Bag X1106, Sovenga 0727, South Africa

(Received 21 February, 2005; accepted 6 September, 2005)

ABSTRACT

Yield stability of white cabbage (*Brassica oleracea* var. *capitata*) cultivars were assessed to identify high yielding and stable cultivar(s) with adequate resistance to black rot caused by *Xanthomonas campestris* pv. *campestris*. Field experiments were conducted with artificial inoculations for three years (1998-2000) in two locations and using 10 selected commercial cabbage cultivar(s). Stability was assessed via joint regression analysis and superiority analysis. The result indicated that cultivars Tenacity, Gideon and Gloria expressed average stability with high yield and adequate resistance. Cultivar Tenacity was selected for its greater mean yield and stability for advanced testing for resistance against black rot disease.

Key Words: *Brassica oleracea*, Ethiopia, stability, *Xanthomonas campestris*

RÉSUMÉ

La stabilité de variétés des choux blancs (*Brassica oleracea* var. *capitata*) était évaluée pour identifier les variétés à haut rendement et stable avec une résistance adéquate to black rot causée par *Xanthomonas campestris* pv. *campestris*. Les expériences de terrain étaient conduites avec des inoculants artificiels pour trois ans (1998-2000) dans trois locations et utilisant 10 variétés commerciales sélectionnées. La stabilité était évaluée en utilisant l'analyse de régression et de supériorité. Les résultants ont indiqué que les variétés Tenacity, Gideon et Gloria exprimées une stabilité moyenne avec haut rendement et une résistance adéquate. La variété Tenacity était sélectionnée pour sa grande moyenne et sa stabilité pour des tests avances de résistance contre black rot.

Mots Clés: *Brassica oleracea*, Ethiopia, stabilite, *Xanthomonas compestris*

INTRODUCTION

White cabbage (*Brassica oleracea* var. *capitata* L) is one of the important vegetable crops grown in Ethiopia (Hussein, 1989). Introduction of improved hybrid seeds of the crop remains essential cultivation practice to the country. This is due to the lack of local breeding firms to supply hybrid or open pollinated cultivars to cultivate the crop. In tropical environments vernalisation requirement cannot be realised to induce flowering and subsequent seed set. Consequently introduced cultivars can be exploited with their spillover over effects for yield and other important traits such as resistance to plant pathogens. In the humid tropics, however, black rot caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pam.) Dawson is devastating to cabbage and its close relatives. This is due to cultivars susceptibility and environmental conditions that favour the pathogen (Staub and Williams, 1972; Ignatov *et al.*, 1998 a,b, 1999).

Multi-environmental trails are important procedure in plant breeding for recommendation and release superior and stable cultivar(s). Such trails allow comparison of mean yield and stability of genotypes, among other tasks. Consequently it is possible to select superior cultivar(s) based on greater yield and average stability. Yield and its stability depend on the genetic constitution of the cultivar and the intensity of the environmental conditions (Bradshaw, 1965; Borojevic, 1990). Thus, to select high yielding and stable cultivar(s) it is essential to test them under the target production environments in a range of growing conditions.

Different stability parameters have been proposed but the choice of any of these methods mainly depend on whether one considers stability over a wide range of environments or the relative stability of a group of cultivars included in a given experiment. The Eberhart-Russel regression analysis (Eberhart and Russel, 1966) and Lin and Binns' superiority parameter (Lin and Binns, 1988) are stability parameters of interest and are among the commonly used stability models. The parameter of Eberhart and Russel (1966) is based on the regression of each genotypic yield on the environmental index (the mean yield at each environment). According to Eberhart and Russel (1966), a stable cultivar has a regression coefficient close to unity (b_i=1), minimum deviation from regression ($\sum s^2 d_i$=0) and high mean yield. Lin and Binns' (1988) superiority parameter (P_i) is the squared difference between cultivar's yield and the maximum yield within each environment, averaged over all environments. Genotypes with broader adaptation have lower values of this superiority parameter, because they yield closer to the maximum within each environment, relative to genotypes with poor adaptation to the target environments.

In Ethiopia, Hussein (1989) described that black rot of cabbage is the most damaging and prevalent disease causing a serious yield reduction in low input farming systems. Losses range from quality deterioration to complete crop failure. Studies were carried at Alemaya University (Ethiopia) and identified promising hybrids of cabbage that had considerable level of resistance to locally available strains of the pathogen (Shimelis and Swart, 2004). The present study was conducted to assess the average yield and yield stability of selected white cabbage cultivars under field conditions and over ranges of test environments. The information is helpful for cultivar recommendation where the locally known cultivar Copenhagen market has been found susceptible for black rot.

MATERIAL AND METHODS

Study sites. The study was conducted at Alemaya and Dire Dawa, both representing main and sub-research stations of Alemaya University (Ethiopia), respectively. Alemaya (coordinates 09°24'N, 42°E) has an elevation of 1980 m above sea level (masl) and receives an average annual rainfall of 893 mm. It has annual maximum temperature ranging from 20-25 °C and minimum 8-13 °C. While Dire Dawa (9°N36', 41°E51') is situated at an altitude of 1176 masl with an annual average rainfall of 612 mm. Dire Dawa has annual maximum temperatures ranging from 25-35 °C and minimum 17-21 °C. Soil, climatic, and biological conditions of the two stations varies considerably.

Plant preparation and artificial inoculation. The study used 10 white cabbage genotypes that were kindly supplied by different seed companies (Table 1). Their level of resistance has been investigated using locally available black rot isolates under glasshouse conditions. Details of the evaluation experiments were given by Shimelis and Swart (2004).

Prior to filed planting seedlings were raised in plastic pots of 250 ml capacity by growing one healthy and strong seedling per pot. At four-leaves growth stage, the seedlings were artificially inoculated with a virulent strain of *X. campestris* pv. *campestris* chosen from earlier virulence studies (Staub and Williams, 1972). Seedlings were artificially inoculated by means of spray and injection. Thirty seedlings per cultivar were available and subjected for each inoculation techniques. Inoculum was prepared at 5 x 108 cells/ml. The bacterium was grown on yeast peptone glucose agar maintained at a pH of 7 and temperature 27 °C for 44 h. The entire surface of each plant was uniformly sprayed using a micro ulva sprayer (Micro Sprayers Ltd., Bromyard, England). Inoculation by means of injection was carried out using a sterile 1 ml hypodermic syringe. The bacterial suspension (0.04 ml) was injected near the base of the leaf close to the petiole. Seedlings were allowed to slowly dry and transplanted to field with a ball of earth. Studies were conducted from July to October 1998, 1999 and 2000.

Establishment of field experiment. The experiment was designed in a randomised complete block design with three replications. The plot size was 7.2 m^2 (3 rows, 0.6m apart and 4m long) with net harvest area of 3.6 m^2. Plants were spaced 60 cm x 60 cm. Diamonium phosphate (21% N, 53% P_2O5) and urea (99% N) were applied at a rate of 30 kg ha^{-1} of which 15 kg ha^{-1} was used during planting and the remaining portion applied at head initiation growth stage. The fertilisers were manually incorporated by hoeing the soil around plants. Supplemental irrigation was applied by hosepipe (Alemaya) and furrows (Dire Dawa) as required.

Data collection, statistical analysis and stability parameters. Data were collected on the weight of marketable head that were harvested from plants free from black rot or showing few lesions only to lower leaves. At harvest heads were collected and weighed individually to get the yield per plot that was later converted to t ha^{-1}. A combined analysis of variance (ANOVA) of yield was conducted across environments using the SAS ANOVA procedure (SAS, 1996). Mean comparisons among cultivars were performed using the LSD procedure at 5% probability level (Steele *et al.*, 1997). To assess yield stability, Eberhart and Russel's (1966) joint regression model was used and the yields of each genotype were regressed on the mean environmental yields. Accordingly a cultivar was considered stable showing a regression coefficient (b_i) close to unity and a deviation from regression (residual variance=$\sum s^2d_i$) close to zero. To compute the superiority index (P_i) (Lin and Binns, 1988) the maximum mean yield among all genotypes were noted at each location. Then for each genotype, the mean square difference between its yield and the maximum yield at that environment was determined. This was repeated for each environment and the mean square difference between the genotype and the maxima was averaged over environments. Stability parameters were computed using SAS (1996) and Agrobase (2000).

TABLE 1. List of cabbage cultivars used in the study indicating predicted and actual levels of resistance and sources

Cultivar	Level of resistance		
	Predicted [a]	Observed[b]	Source (country/breeding firm)
Comsa	Tolerant	S	Denmark/Daehnfeldt
Gloria	Tolerant	MR	Denmark/Daehnfeldt
Riana	Tolerant	S	Denmark/Daehnfeldt
Rotan	Tolerant	S	Denmark/Daehnfeldt
Fresco	Susceptible	HS	The Netherlands/Bejo Zaden[b.v.]
Bronco	Tolerant	MS	The Netherlands/Bejo Zaden[b.v.]
Gideon	Tolerant	MR	The Netherlands/Bejo Zaden[b.v.]
Tristar	Tolerant	MS	The Netherlands/Bejo Zaden[b.v.]
Tenacity	Highly tolerant	R	The Netherlands/Sluis and Groot
Copenhagen market	Susceptible	HS	Denmark/Daehnfeldt

[a] Cultivar resistance described as tolerant/highly tolerant/susceptible by the parent seed companies
[b] Studies conducted in Ethiopia (Shimelis and Swart, 2004): R (Resistant) (1-2 necrotic lesions present/leaf measuring $\leq$ 5 mm^2); MR (moderately resistant) (3-4 lesions, 6-25 mm^2); MS (moderately susceptible) (5-6 lesions, 26-45 mm^2); S (susceptible) (7-8 lesions, 46-65 mm^2); HS (highly susceptible) ($\geq$ 9 necrotic lesions, $\geq$ 66 mm^2)

RESULTS

Combined analysis of variance. The result of combined ANOVA on yield of genotypes over environments after spray and injection methods of testing is summarised in Table 2. The analysis following spray inoculation indicated that there were significant differences among genotypes (g), g x locations (l) as well as g x l x years (y) interaction. However, no significant differences were detected between y, l, and g x y interaction. A further analysis using injection inoculation suggested that there were significant differences among g and l main effects. The results from both analyses allowed stability studies of genotypes over environments.

Cultivar performance versus environments. The mean yield of genotypes across six environments is summarised in Table 3. The average yield over years at Alemaya and Dire Dawa indicate that cultivars Tenacity, Gideon and Gloria were relatively the best performers. At Alemaya, following spray inoculation Gideon ranked first (64.96 t ha^{-1}) followed by Tenacity (64.45 t ha^{-1}) and Gloria (53.62 t ha^{-1}). However, after injection inoculation Tenacity ranked first (59.18 t ha^{-1}) followed by Gideon (58.43 t ha^{-1}) and Gloria (46.74 t ha^{-1}). At Dire Dawa, Tenacity was the best performer yielding 63 t ha^{-1} and 58.18 t ha^{-1} following spray and injection means of testing, respectively. At this location, Gideon ranked 2nd and Gloria 3rd after both inoculations.

When the two locations were compared the average yield levels of genotypes were relatively better at Alemaya than Dire Dawa. This might have been attributed to higher temperature conditions favourable to the pathogen inflicting greater yield reductions at Dire Dawa. During the experiments, at Dire Dawa, the average daily maximum temperature varied from 31-33 °C and the minimum 19-21 °C. While at Alemaya the daily maximum temperature were from 22-25 °C and the minimum 9-13 °C. Due to black rot intensity premature plant deaths were common at Dire Dawa. In both locations it was noted that

TABLE 2. Combined analysis of variance of yield of 10 white cabbage genotypes[a]

Source of variation	DF	Mean square	F Value
Spray			
Genotype (g)	9	9523.32	1561.91**
Year (y)	2	6.39	1.05
Location (l)	1	22.26	3.65
g x y	18	4.88	0.80
g x l	9	14.44	2.37*
g x y x l	18	14.53	2.38**
Replication in l*y	12	18.952	3.11**
Error	110	6.09	
Injection			
G	9	8307.05	875.01**
Y	2	14.96	1.58
L	1	39.87	4.20*
g x y	18	2.79	0.29
g x l	9	8.01	0.84
g x y x l	18	15.11	1.59
Replication in l*y	12	4.54	0.48
Error	110	9.49	

[a] Tests were done using spray and injection methods of infection by *Xanthomonas campestris* pv. *campestris* for three years and two locations using three replications

* and ** denote significant differences at 0.05 and 0.01 levels of probability, respectively

TABLE 3. Yield (t ha^{-1}) of ten cabbage genotypes when tested for three years (1998-2000) and two locations (Alemaya and Dire Dawa) using spray and injection methods of infection by *Xanthomonas campestris* pv. *campestris*

Genotype	Year	Location and test method			
		Alemaya		Dire Dawa	
		Spray	Injection	Spray	Injection
Comsa	1998	25.68	20.20	25.07	21.61
	1999	25.66	21.05	24.85	17.03
	2000	25.74	20.68	25.02	19.38
	Mean	25.69	20.64	24.98	19.34
Gloria	1998	55.78	48.78	51.33	44.50
	1999	51.33	44.50	54.79	46.20
	2000	53.75	46.94	53.25	45.65
	Mean	53.62	46.74	53.12	45.45
Riana	1998	14.00	9.24	10.65	7.76
	1999	10.65	7.76	10.93	7.59
	2000	12.37	8.53	10.84	7.71
	Mean	12.34	8.51	10.81	7.69
Rotan	1998	13.94	9.65	12.07	8.32
	1999	12.07	8.32	17.43	11.00
	2000	13.04	9.02	14.79	9.70
	Mean	13.02	9.00	14.76	9.67
Fresco	1998	8.67	7.83	6.07	6.00
	1999	6.07	6.00	10.68	9.52
	2000	7.39	6.94	8.40	7.78
	Mean	7.38	6.92	8.38	7.77
Bronco	1998	19.50	15.65	20.45	14.17
	1999	20.45	14.17	17.56	12.63
	2000	20.02	17.97	19.05	13.46
	Mean	19.99	15.93	19.02	13.42
Gideon	1998	64.20	56.44	65.63	60.37
	1999	65.63	60.37	56.87	49.31
	2000	65.06	58.49	61.39	54.93
	Mean	64.96	58.43	61.30	54.87
Tristar	1998	18.19	13.02	16.93	12.09
	1999	16.92	12.09	12.91	9.28
	2000	17.62	12.58	14.98	10.71
	Mean	17.58	12.56	14.94	10.69
Tenacity	1998	63.78	58.24	65.07	60.08
	1999	65.07	60.07	60.76	56.22
	2000	64.50	59.24	63.18	58.23
	Mean	64.45	59.18	63.00	58.18
Copenhagen Market	1998	4.43	4.39	4.48	4.22
	1999	4.48	4.22	7.90	5.26
	2000	4.46	4.32	6.20	4.76
	Mean	4.46	4.31	6.19	4.75

yield level was considerably lower when plants were subjected to injection than spray inoculation (Table 3). Thus, testing by injection was detrimental to yield that might be confounded from artificial wounding of plants.

Stability parameters. The over all mean yield of genotypes and the joint regression and superiority stability statistics are presented in Table 4. When cultivars were compared using mean yield performance Tenacity ranked 1st, Gideon 2nd and Gloria 3rd for both inoculations. Except Tenacity and Gideon following both inoculations as well as Riana, Rotan and Fresco during injection inoculation there were significant differences for yield between genotypes. The locally grown cultivar Copenhagen Market gave the lowest yields and ranked last in both test conditions.

According to the joint regression stability model cultivar Tenacity displayed average stability followed by Gideon and Bronco. These cultivars had a regression coefficient (b_i) closer to 1 and low residual variance ($\Sigma s^2 d_i$) (Table 4). Copenhagen Market, Rotan, and Fresco were sensitive indicating considerable yield variations over environments. Tristar and Copenhagen Market showed a significant deviation from linearity after injection inoculation.

The superiority parameter allocated cultivars Tenacity, Gideon and Gloria with average stability (Table 4). Cultivars ranking using the superiority parameter were similar to ranks obtained using average yield performance. Genotypes with low yield had higher P_i and ranked furthest (Table 4).

In general the two stability models identified Tenacity, Gideon and Gloria as the most stable genotypes. Tenacity had maximum and stable yield performance across test environments. It was thus possible to recommend Tenacity as a promising resistant genotype that can be exploited in environments where black rot is prevalent in Ethiopia.

Spearman's rank correlation analysis showing the association of cultivars ranks following mean yield and the two stability parameters is presented in Table 5. The result suggested that ranks obtained using these parameters were positively correlated to each other. Furthermore, there were strong significant correlations between average yield and superiority index (Table 5).

TABLE 4. Mean yield (t ha^{-1}) and joint regression and cultivar superiority stability statistics for comparisons of ten cabbage genotypes tested by spray and injection methods of infection using *Xanthomonas campestris* pv. *campestris*[a]

Genotype	Spray inoculation							Injection inoculation							Over all
			Joint regression			Superiority				Joint regression			Superiority		
	Mean yield[b]	R	b_i	$\Sigma s^2 d_i$	R	P_i	R	Mean yield[b]	R	b_i	$\Sigma s^2 d_i$	R	P_i	R	R
Comsa	25.33c	4	0.44	-2.27	4	731.72	4	19.99c	4	1.98	-2.28	6	752.23	4	5
Gloria	53.37b	3	2.39	-1.42	5	75.61	3	46.26b	3	0.65	-0.74	3	81.01	3	3
Riana	11.57g	8	0.79	-2.55	4	1295.29	8	8.09f	8	0.62	-2.77	5	1285.47	8	7
Rotan	13.89f	7	2.20	-1.44	6	1211.31	7	9.33f	7	-1.04	-2.47	7	1225.26	7	7
Fresco	7.88h	9	-0.18	2.95	7	1353.40	9	7.34f	9	-1.34	-1.99	8	1326.19	9	8
Bronco	19.50d	5	0.93	-2.74	3	992.82	5	14.18d	5	1.38	-2.80	2	995.64	5	4
Gideon	63.13a	2	0.98	-2.83	2	2.49	2	56.65a	2	4.84	3.45	4	5.20	2	2
Tristar	16.26e	6	0.35	-2.53	4	1119.33	6	11.63e	6	1.91**	-3.04	3	1112.19	6	6
Tenacity	63.76a	1	1.12	-2.86	1	0.01	1	58.68a	1	1.52*	-1.92	1	0.01	1	1
Copenhagen market	5.33i	10	0.9	-2.75	4	1489.45	10	4.53g	10	-0.52*	-3.02	9	1473.07	10	9
LSD (P=0.05)	1.65							2.05							

a R=rank; b_i=regression coefficient; $\Sigma s^2 d_i$=sum of deviation from regression; b means in a column followed by the same letter are not significantly different.
* and ** denote significant difference from linearity at P=0.05 and P=0.01 respectively

DISCUSSION

Cultivars Tenacity, Gideon and Gloria were identified as potentially high yielding possessing adequate resistance to black rot when compared to a locally grown susceptible cultivar Copenhagen Market. Among the tested cultivars Tenacity was the best both in terms of yields and yield stability. This observation suggest maximum heterozygosity for cultivar Tenacity. In adverse conditions heterozygotes have been reported better performers than their homozygous parents (Allard and Workman, 1963; Griffing and Langridge, 1963). Hybrid cultivars would have high genetic yield potential as well as high adaptability and yield stability. The cultivars are genetically homogenous but heterozygous, the basis for full expression of their physiological homeostasis (Borojevic, 1990).

The present study attempted and utilised average yield performance and stability models to establish black resistance of selected cabbage cultivars. Admittedly, results obtained after injection inoculation rendered poor G x E interaction indicating the weakness of the stability analysis. Therefore, in black rot resistance trials it would be appropriate to carry out selection after spray or injection inoculation techniques based on average yield performance across adequate environments. Selections based on average yield level provided consistent ranking of cultivars showing strong correlation with results of the stability analysis. However, for both inoculation techniques cultivar Tenacity is the most stable and high yielding genotype in various environments in Ethiopia. However, essential horticultural attributes of the cultivar need to be investigated to make a specific recommendation and subsequent large scale production in black rot problem areas in the country.

TABLE 5. Spearman's rank correlation coefficients showing association of cultivars ranks following average yield (X1) and joint regression (X2) and cultivar's superiority (X3) stability parameters

	X1	X2	X3
X1[a]	1	0.77**	1.0**
X2	0.61	1	0.77**
X3	1.0**	0.61	1

[a] Correlation coefficients at lower and upper diagonals estimated after spray and injection methods of testing, respectively

** Correlation is significant at 0.01 level of probability

ACKNOWLEDGEMENT

The Government of Ethiopia is sincerely acknowledged for providing financial support to Alemaya University through the Ethiopian Agricultural Research Organization. Mr. Shimelis Fleleke, Ms. Haimanot and Kokebie are thanked for their technical assistance and data collection.

REFERENCES

Agrobase, 2000. *Users Guide and Reference Manual*. Agronomix Software, INC., Manitoba, Canada.

Allard, R.W. and Workman, P.L. 1963. Population studies in predominantly self-pollinated species. IV Seasonal fluctuations in estimated values of genetic parameters in lima bean populations. *Evolution* 17:470-480.

Borojevic, S. 1990. *Principles and Methods of Plant Breeding*. Elsevier, Amsterdam.

Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* 13:115-155.

Eberhart, S.A. and Russell,W.A. 1966. Stability parameters for comparing varieties. *Crop Science* 6:36-40.

Griffing, B. and Langridge, J. 1963. Phenotypic stability of growth in the self fertilized species *Arabidopsis thaliana*. In: *Statistical Genetics in Plant Breeding*. National Academy of Sciences, *National Research Council Publication* 982:386-394.

Hussein, T. 1989. Prevalence, distribution, biology and control of black rot in Hararghe, Ethiopia. Crop protection annual research report, Alemaya University. pp.17-24.

Ignatov, A., Kuginuki, Y. and Hida, K. 1998a. Race-specific reaction of resistance to black rot in *Brassica oleracea*. *European Journal of Plant Pathology* 104:821-827

Ignatov, A., Hida, K. and Kuginuki, Y. 1998b. Black rot of crucifers and sources of resistance in Brassica crops. *Japan Agricultural Research Quarterly* 32:167-172.

Ignatov, A., Kuginuki, Y. and Hida, K. 1999. Vascular stem resistance to black rot in *Brassica oleracea*. *Canadian Journal of Botany* 77:442-446.

Lin, C.S. and Binns, M.R. 1988. A superiority measure of cultivar performance for cultivar x location data. *Canadian Journal of Plant Science* 68:193-198.

SAS Institute. 1996. SAS/IML software: Usage and reference. SAS Institute, Cary.

Shimelis, H.A. and Swart, W.J. 2004. Study of black rot development in introduced cabbage hybrids in Ethiopia. *African Plant Protection* 10:43-52.

Staub, T. and Williams, P.H. 1972. Factors influencing black rot lesion development in resistant and susceptible cabbage. *Phytopathology* 62:722-728.

Steele, R.D.G., Torrie, J.H. and Dickey, D.A. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. McGraw-hill, New York. 665pp.