Aust. J. Agric. Res., 1994, **45**, 65–74

Evaluation of Pre-Harvest Sprouting in Triticale Compared with Wheat and Rye Using a Line Source Rain Gradient

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Abstract

Field grown triticales, wheats and ryes were subjected to an artificial rain gradient with the aim of discriminating among different genotypes on the basis of their tolerance to wetting, measured as change in falling number (FN) and alpha-amylase activity (AA). Below-canopy gravity irrigation throughout the growing cycle ensured stress-free conditions. The rain gradient was created by overhead irrigation from a central water line or line source. Plots were sown perpendicular to the line source and sampled for AA throughout the gradient and over sampling dates. The gradient was also analysed for FN 30 days post physiological maturity (PM+30). Based on change in AA over both the gradient and sampling dates and change in FN at PM+30, genotypes tolerant to wetting were identified. The factors most important in conferring tolerance to wetting among triticales were spike angle, thousand-grain weight and FN levels. Whilst the line source offered good discrimination among triticales on the basis of change in FN and AA, it was not effective in distinguishing between wheat and rye lines.

Keywords: line source, rain gradient, triticale, falling number, alpha-amylase, seed dormancy, bract inhibitors.

Introduction

Preharvest sprouting is a problem encountered by triticales when sown for flour production in regions where moist, humid conditions prevail during harvest. The mechanisms responsible for pre-harvest sprouting tolerance (PST) among a subset of CIMMYT (International Maize and Wheat Improvement Centre) triticales have been studied (Trethowan *et al.* 1993). Seed dormancy, falling number (FN) levels, and chemical and mechanical bract related factors were found to influence the level of PST. To successfully breed for PST, it is necessary to both identify variation for individual components of tolerance and develop methods by which this variation can be combined.

The CIMMYT triticale program shuttles breeding material between rainfed locations in the Central Mexican Highlands and an arid, irrigated location in north western Mexico. The aim of this study was to identify variation for wetting tolerance, measured as change in FN and AA, and relate this to the presence or absence of different components of PST in triticales, wheats and ryes grown under rain gradients generated by line source overhead irrigation in northern Mexico.

Materials and Methods

Treatments and Observations

A set of twelve complete triticales (all seven rye chromosome pairs present), eight substituted triticales (2D/2R substitution), six primary triticales and their wheat (six) and rye (six)

0004-9409/94/010065$05.00

progenitors were sown in a three replicate randomized complete block design at Ciudad Obregon in north-western Mexico (lat. 19° N., 60 masl). The complete and substituted triticales had been previously tested for sprouting tolerance and were selected for their variable expression of tolerance (Trethowan *et al.* 1993). Double row plots of 15 m length were sown perpendicular to the line source, an overhead irrigation system of equally spaced sprinkler nozzles on a central water line.

Throughout the growth cycle below canopy gravity irrigation ensured moisture stress-free conditions. Overhead sprinkler irrigation was applied on average every second night from the onset of anthesis and continued until 30 days post physiological maturity (PM+30). Physiological maturity was determined as loss of chlorophyll from the peduncle 15 cm below the spike. Each plot was divided into six sampling zones each of 1·5 m located at 2 m intervals from the line source. The sixth zone located furthest from the water line received no overhead water. Water application followed a linear response. Fifteen seeds were taken from one of three central spikelets from 15 different spikes within each zone at six different dates. Sampling dates were 15 and 30 days post-anthesis, PM, PM+10, PM+20 and PM+30. Samples were freeze-dried and kept in the freezer until assayed for AA activity.

At PM+30 each sampling zone in the line source gradient was bulk harvested and tested for FN (AACC 1983).

Spike angle and plant height were scored at PM, upright spikes received a score of 1 and those with the central spikelets completely inverted were rated as 5. Thirty spikes were selected from the water free zone (zone 6) at PM+10. To determine the level of seed dormancy expressed in this environment, 20 seeds were hand threshed from five spikes and germinated along with reserve (non-dormant) seed samples on petri dishes at 30°C. Per cent germination was determined daily for 7 days. To examine the effects of water-soluble bract inhibitors 15 spikes were threshed and half the resultant seed treated with exudates made from ground chaff using the methods outlined by Trethowan *et al.* (1993), the remaining seed was germinated in distilled water. The remaining spikes from each plot were soaked in water for 18 h, wrapped in moist paper towelling, banded in plastic and incubated at 22°C for three days. Details of the levels of seed dormancy, bract inhibition and intact spike germination have been presented earlier (Trethowan *et al.* 1993).

Alpha Amylase Activity

Two malted barley samples were obtained from Extractos y Maltas S.A., Mexico, D.F., Mexico, for the determination of AA activity. Samples were ground and assayed colorimetrically for AA activity using dye-labeled amylase substrate marketed as Phadedas tablets (Pharmacia Diagnostics A B Sweden), using the procedure described by Mathewson and Pomeranz (1977). Nine millilitres of sodium phosphate (pH 6·2) buffer solution (Mathewson *et al.* 1982) were added to the sample (50 to 100 mg) contained in a plastic bottle (300 × 6·5 mm), vortex mixed and heated at 50°C in a water-bath. Shaking ensured thorough suspension during the enzymatic reaction time. Following preheating (4-min), one Phadebas tablet was added to the bottle, vortex mixed and returned to the water bath for a 4-min enzymatic reaction period. The reaction was stopped by adding 1·0 mL of 0·5 M NaOH to the reaction bottle. After cooling, the coloured fluid was separated from the reaction mixture by centrifuging for 5-min at 7000 rpm (6000 × g). Absorbance of the supernatant was determined at 620 nm. The relationship between absorbance at 620 nm and AA activity in millidextrinizing units (mDU/g) (American Society of Brewing Chemists 1958) was established by preparing a standard curve using the two malted barley samples. The resulting regression equation was $y = 32{\cdot}88(x) + 2{\cdot}48$, where x=absorbance at 620 nm and y = mDU. The correlation coefficient (r) was 0·994.

Results and Discussion

Effectiveness of the line Source Gradient in Detecting Changes in AA and FN

Preliminary tests for AA activity demonstrated that the first two, middle two and last two sampling zones gave equivalent information. All subsequent tests

Table 1. The relationship between FN and AA activity from the line source gradient, date 3

FN/AA	Zone 1	Zone 2	Zone 3
Zone 1	−0·68***	−0·61***	−0·71***
Zone 2	−0·60***	−0·63***	−0·73***
Zone 3	−0·64***	−0·58**	−0·63***

$**P < 0·01$; $***P < 0·001$.

were then carried out on zones 1, 3 and 5 and will now be referred to as zones 1 (high rainfall), 2 (intermediate rainfall) and 3 (no rainfall), respectively. Dates 1, 3 and 5 similarly provided best discrimination and are henceforth referred to as dates 1 (anthesis+15 days), 2 (PM) and 3 (PM+20). Correlation coefficients (r) for comparisons between FN and AA activity were -0·68***, -0·63*** and -0·63*** for zones 1, 2 and 3, respectively (Table 1). Wheats were not included in the calculation of r as FN values clustered at the extreme range of the test. The strongest correlations were observed for zone 3 comparisons, indicating a slight divergence in the relationship of these two tests as spike wetting increased.

Table 2. Change in AA activity across sampling zones and dates for all species and triticale separately

Means within rows followed by the same lower case letter are not significantly different at $P < 0·05$. Means within columns followed by the same upper case letter are not significantly different at $P < 0·05$

	All species			Triticale Only		
	Zone 1	Zone 2	Zone 3	Zone 1	Zone 2	Zone 3
Date 1	0·93aA	0·94aA	0·93aA	0·87aA	0·91aA	0·89aA
Date 2	0·08aB	0·09aB	0·06aB	0·09abB	0·11aB	0·07bB
Date 3	0·18aC	0·16aC	0·08bB	0·24aC	0·22aC	0·10bB

Alpha-amylase activity varied across sampling dates and zones (Table 2). No change in AA occurred between the high and low rainfall zones at PM, however, differences were significant at PM+20 ($P < 0·01$). Differences between PM and PM+20 for AA were significant at high and intermediate rainfall levels and non-significant in the absence of wetting. Results indicate that discrimination among genotypes for change in AA is possible across sampling zones and dates. However, no visible sprouting was observed in the high rainfall zone.

Differences among Species and Biotypes for AA and FN under the Line Source Gradient

AA and FN responses were variable within sampling zones (Table 3). Complete and substituted triticales were not significantly different in either test. Wheat and rye were significantly superior to complete and primary triticales, and wheat superior to substituted triticales in both tests. Tests between complete and primary triticales, substituted and primary triticales, substituted triticales and rye and wheat and rye all showed non-significance in one of the tests. Generally, primary triticales were inferior to all other biotypes and species, complete and substituted triticales performed similarly, and wheat and rye were better than all triticales.

Table 3. Comparisons among species and triticale biotypes for AA and FN for each sampling zone, date 3

Means within columns followed by the same letter are not significant at $P < 0{\cdot}05$. CTCL, STCL and PTCL refer to complete, substituted and primary triticales, respectively

	AA activity mDU/g			FN s		
	Zone 1	Zone 2	Zone 3	Zone 1	Zone 2	Zone 3
CTCL	0·20a	0·23a	0·09a	316a	342a	408a
STCL	0·13a	0·12a	0·07a	312ad	334ad	486a
PTCL	0·53ac	0·35a	0·18a	113b	152b	230b
WHEAT	0·04c	0·04b	0·03b	905c	955c	994c
RYE	0·04c	0·04b	0·04b	401d	426d	441a

Using the Line Source Gradient to Identify Wetting Tolerant Genotypes

The magnitude of change in AA activity between dates 2 and 3 and zones 1 and 3 for each genotype give an indication of the effectiveness of tolerance (Fig. 1 and 2). Little difference between zones was evident at PM, however, by PM+20 substantial increases in AA were evident (Fig. 1). AA activity among some genotypes (1, 3, 4, 5, 7, 8, 10, 11 and 12) did not change appreciably, indicating tolerance; others (13, 22, 24, 25, 27, 29 and 37) showed significant increases in AA and are therefore classified as susceptible. Change in AA activity with spike wetting does not appear to be related to the magnitude of AA activity in untreated spikes. Genotypes 23, 30, 32 and 35 all show high AA activity in zone 3, yet did not change appreciably with wetting (zone 1). Conversely, genotypes 13, 22, 24, 25 and 27 showed lower zone 3 levels of AA, however, produced significantly more AA with increased spike wetting.

Differences between PM and PM+20 days for AA activity were less in zone 3 than the high rainfall zone 1 (Fig. 2). The fact that some genotypes in the rain free zone 3 (32, 33, 35 and 37) produced significantly more AA at PM+20 suggests the synthesis of late maturity AA, a phenomenon independent of rainfall (Mares and Oettler 1991). Much larger differences in AA activity between PM and PM+20 days were evident for some genotypes (13, 25, 27 and 37) from zone 1. Wetting tolerant genotypes (1, 2, 4, 5, 7, 8, 9, 10, 11) showed no change in AA between the two dates.

Change in FN between zones 1 and 3 indicated that certain genotypes (8, 22, 24, 29, 31, 34 and 35) were more susceptible to spike wetting than others (1, 3, 4, 5, 7, 11, 1213) regardless of the zone 3 FN level (Fig. 3).

There were some discrepancies between FN and AA identification of tolerant and intolerant genotypes, which relates to the magnitude of correlation ($-0{\cdot}63$***) between these two tests (Table 1). However, shortcomings in the colorimetric test for AA activity are balanced by the two-dimensional nature of the data collected. Change in AA was observed across both dates and sampling zones, allowing verification of an individual genotypes response to spike wetting. Twenty seven genotypes from a total of 38 were identified as having tolerance to rain based on lack of change in AA and/or FN across both dates and zones for all species and biotypes (Table 4). Among the 26 triticale biotypes tested, 15 were similarly identified.

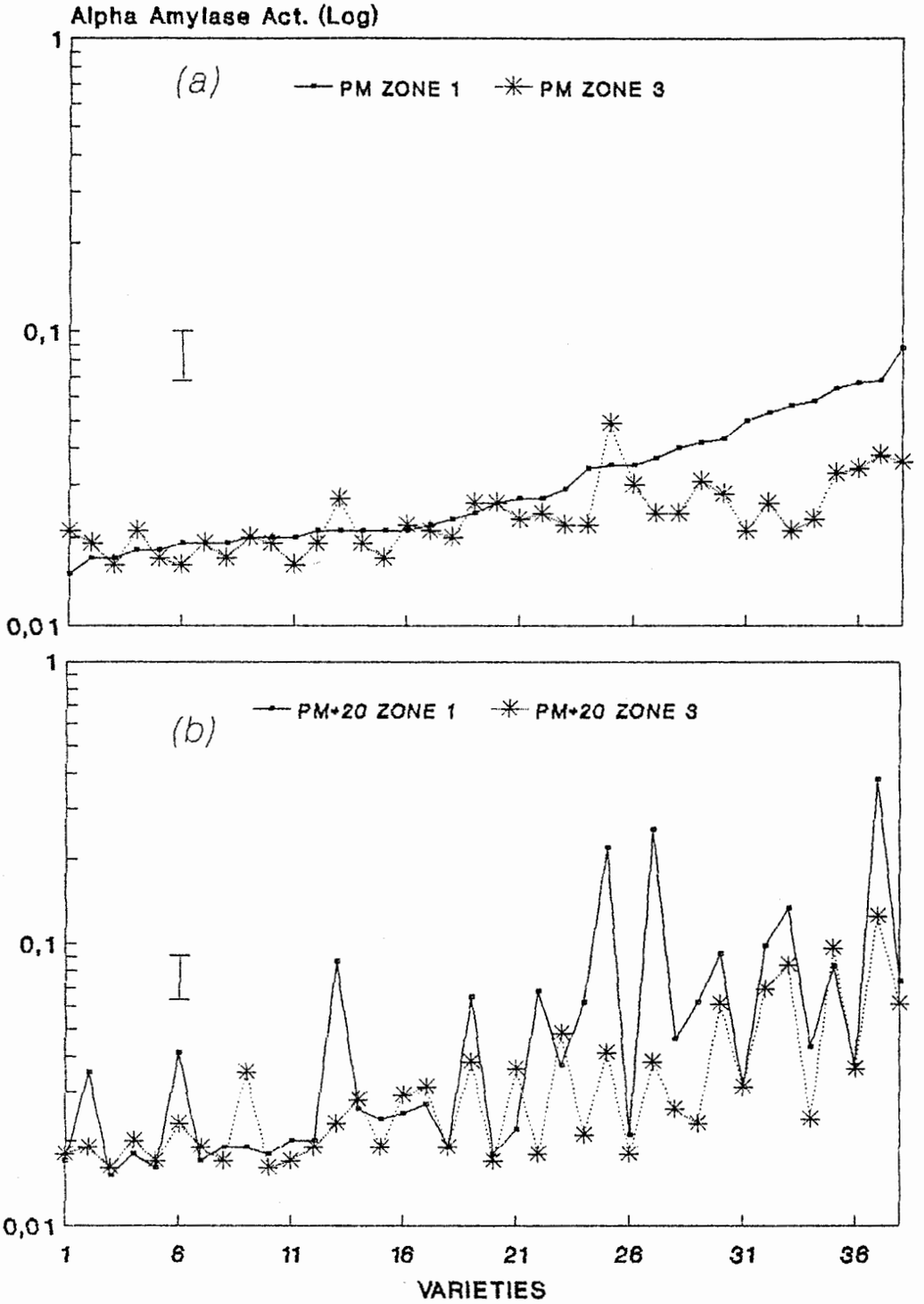

Fig. 1. Comparison of alpha-amylase activity in Zones 1 and 3 at PM and PM+20.

The influence of Agronomic, Morphological and Biochemical Traits on the Expression of FN and AA Activity

Plant height was negatively correlated with FN across all sampling zones (Table 5). This response was consistent across all species and biotypes (averaging

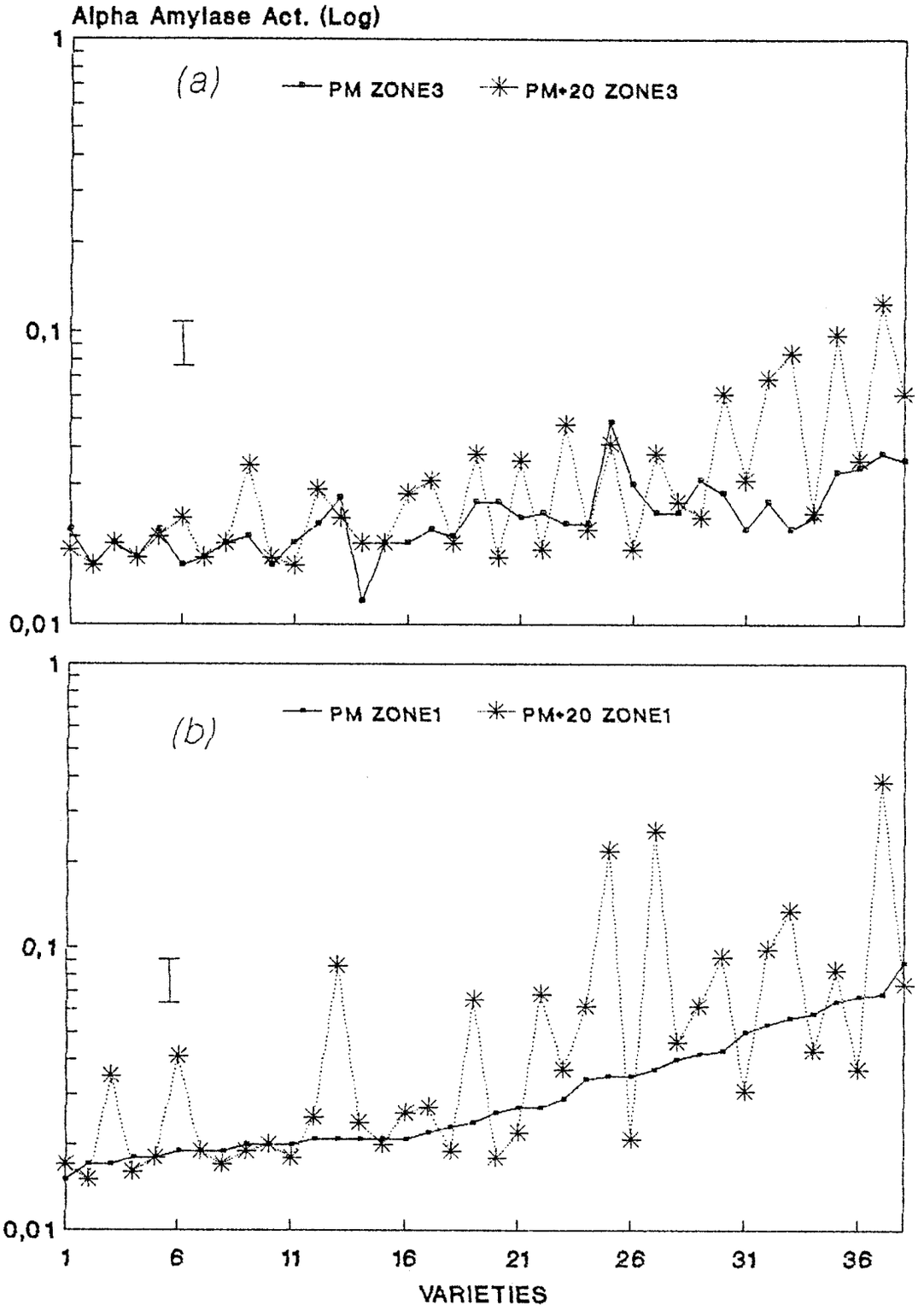

Fig. 2. Comparison of alpha-amylase activity at PM and PM+20 in Zones 1 and 3.

$-0{\cdot}45$) and for comparisons with triticale only ($-0{\cdot}52$). The result is supported by the significantly positive association between plant height and per cent germination in intact spikes over all species and biotypes ($0{\cdot}34$*) and triticales ($0{\cdot}47$*) only, respectively.

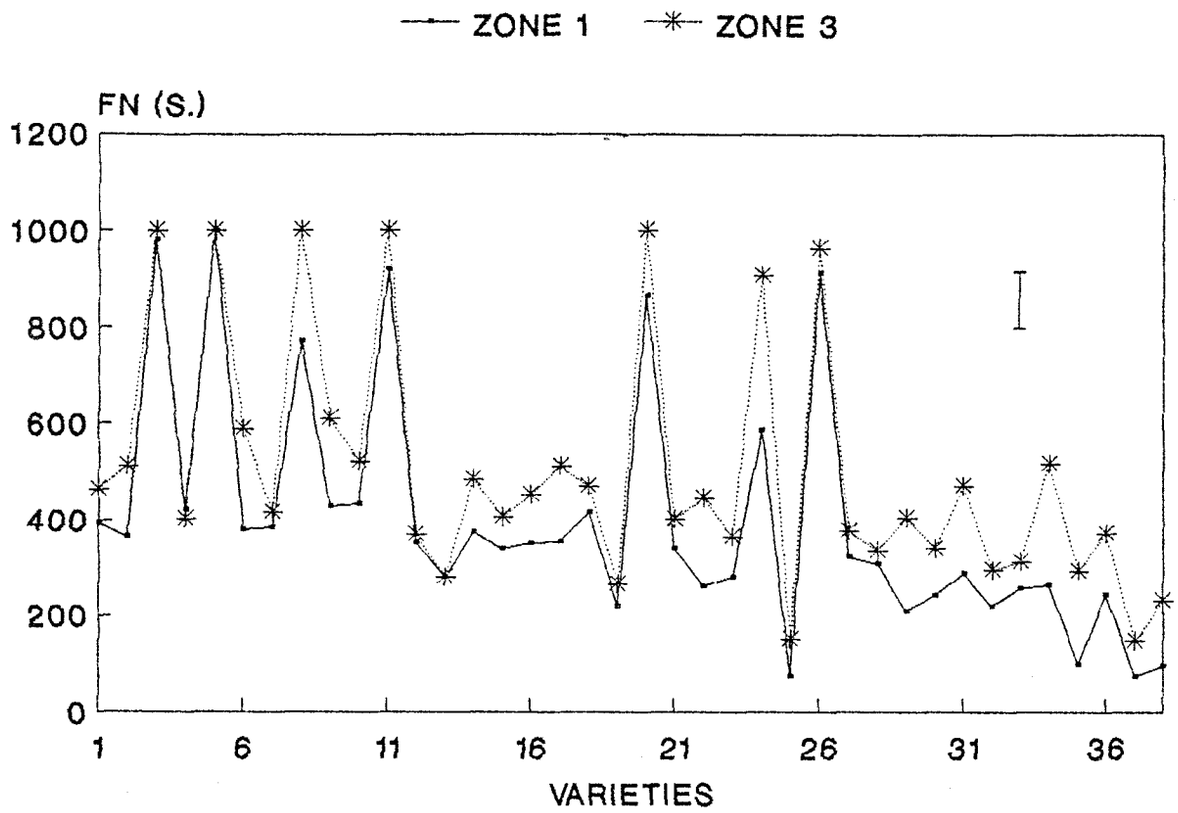

Fig. 3. Comparison of falling number in Zones 1 and 3 at PM+30.

Table 4. Biotype and species identification for each of the 38 genotypes tested for AA activity and FN from the line source gradient

Biotype/ species[A]	Tolerant based on Change in FN and/or AA	No tolerance
CTCL	13,14,15,16,17,19,21, 27,23	2,9,30,33
STCL	28,31,36	6,24,29,32,34
PTCL	25,35,38	22,37
Wheat	3,5,8,11,20,26	
Rye	1,4,7,10,12,18	

[A] CTCL, STCL and PTCL refer to complete, substituted and primary triticales, respectively.

However, the magnitude of correlation in triticale may be confounded by differences among primary, complete and substituted triticale biotypes for height (primary: 130 cm > complete; 118 cm > substitute; 100 cm) and FN (primary; 230 s < complete; 408 s < substitute; 486 s).

Fast rates of grain fill (determined as thousand-grain weight/days of grain fill) and later flowering in triticale tended to result in reduced FN (averaging $-0{\cdot}47$ and $-0{\cdot}41$, respectively). This may be explained by the high grain ripening temperatures characteristic of this environment, which tend to reduce the grain-filling period in late-flowering types. Higher thousand-grain weight in triticales was associated with lower FN (averaging -0·49). This relationship

Table 5. The influence of various agronomic, morphological and biochemical traits on FN from the line source gradient

Trait[A]	FN Zone 1		FN Zone 2		FN Zone 3	
	ALL	TCL	ALL	TCL	ALL	TCL
PH	−0·44**	−0·54**	−0·40*	−0·43*	−0·51***	−0·58***
FL	−0·21	−0·40*	−0·22	−0·41*	−0·25	−0·41*
SA	−0·22	−0·50**	−0·25	−0·44*	−0·23	−0·35
TGW	−0·24	−0·55**	−0·23	−0·47**	−0·18	−0·46*
RGF	−0·21	−0·53**	−0·21	−0·48**	−0·15	−0·42*
PG	−0·39*	−0·13	−0·38*	−0·15	−0·42**	−0·24
Dormancy	0·20	0·34	0·17	0·27	−0·09	0·37*
BI	−0·06	0·38*	−0·09	0·28	−0·08	0·31

*$P < 0·05$, **$P < 0·01$; ***$P < 0·001$.
[A]PH, FL, SA, TGW, RGF, PG and BI are plant height, flowering date, spike angle, thousand grain weight, rate grain fill, per cent germination and bract inhibitor response, respectively.

is probably strengthened by larger seed types (with lower FN) among primary triticales; it may also relate to the ineffectiveness of the glumes in covering and preventing water entry in larger seed.

FN was negatively associated with upright spikes in the high rain fall zone 1 (-0·55**). This result is difficult to explain as past experience suggests that drooping spikes may shed water better, although this is still to be resolved (King 1989). One explanation may be that substituted triticales have in general higher FN (486 s) than completes (408 s) and have a tendency to produce more upright spikes. Per cent germination in intact spikes and FN were significantly negatively correlated when examined over all species and biotypes (averaging -0·40), however, no significant association existed for triticales alone (−0·21). Factors other than FN levels appear to influence germination among triticales. Comparisons of the above agronomic and morphological traits with AA activity did not, in general, yield significant results.

Information regarding the levels of seed dormancy, bract chemical inhibition and FN levels among these materials have been established (Trethowan *et al.* 1993). However, their influence on intact spike germination and change in FN in combination with other morphological and agronomic traits is now discussed for triticale only, as little or no change in FN or AA was noted for wheat and rye. Standard partial regression coefficients showed that spike angle ($P < 0·01$), thousand-grain weight ($P < 0·05$) and FN level under rain-free conditions ($P < 0·05$) contributed most to estimated change in FN (Table 6). Primary triticales have on average larger seed (thousand grain weight 49) than completes (42) and substitutes (37), and are characterized by lower FN levels (56% of complete triticale), thereby highlighting the influence of thousand grain weight on wetting tolerance. Seed dormancy and bract inhibitors were the least important in their effect on change in FN.

Per cent germination was determined by artificially wetting spikes immediately after harvest, therefore spike angle is not considered in the regression analyses. The only significant contributions to reduced sprouting in intact spikes of triticale were plant height ($P < 0·05$), bract inhibitor ($P < 0·05$) and maturity ($P < 0·05$). Tall early-maturing plants tended to show more sprouting than short late plants;

Table 6. Standard partial regression coefficients for Change in FN and percent germination in intact spikes of triticale

Dependent variable	Independent variable	Std partial reg. coeff.
Change in FN	Spike angle	−0·5949**
	TGW[A]	0·5260*
	FN zone 3	0·4237*
	Dormancy	−0·1576
	Bract inb.	−0·1413
	r^2	0·459
Per cent germination	Plant height	0·5401*
	Maturity	−0·3429*
	Bract inb.	−0·3652
	Rate grain fill	−0·3008
	r^2	0·366

*$P < 0·05$, **$P < 0·01$.
[A] TGW refers to thousand-grain weight.

however, the confounding effects of maturity were controlled by sampling plots a fixed number of days after PM from the rain-free end of the gradient. Spikes were then treated to controlled artificial wetting. The significant association with plant height relates to height differences among the three triticale biotypes. Primary triticales (130·3 cm) were on average taller than completes (118·1 cm) and substituted (100·0 cm) types. Overhead watering was conducted at night followed by relatively high day temperatures. These wetting/drying cycles almost certainly lessened the impact of seed dormancy on wetting tolerance (Mares 1989).

Conclusion

Overhead line source watering began on a regular basis from the onset of anthesis. Each genotype received the same amount of water at the same stage of development, thereby removing the confounding effects of variable rainfall and variable maturity dates characteristic of field screening under natural conditions. Results demonstrated that overhead wetting was effective in producing a gradient for AA activity among triticales. Individual genotypes could be classified as tolerant or intolerant to wetting, and those factors contributing to tolerance in this environment determined. However, the triticales in the present study are generally less tolerant to wetting than wheat and rye. The test was not effective in distinguishing between wheats and ryes where the overall level of tolerance was greater. A lack of aerial humidity in this environment during the day inhibited the effective activation of AA activity among some genotypes. Seed dormancy was not a major contributor to wetting tolerance in the Ciudad Obregon environment where conditions were not conducive to its expression.

Acknowledgments

The authors thank Dr D.J. Mares for his advice and helpful discussion on various aspects of pre-harvest sprouting tolerance.

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Manuscript received 5 June 1993, accepted 26 July 1993