Proc. Indian natn. Sci. Acad. B **45**, No. 3, pp 247-254 (1979)

X-Ray Diffractometric Technique for Screening High Temperature-Resistant Wheat Varieties

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The crystallinity values for several varieties of wheat leaves were obtained by the X-ray diffractometric technique and correlated with their respective thermal injury values evaluated at 40°C by tissue conductivity method. The X-ray crystallinity of the leaf dry matter showed a high negative correlation ($r=-0.7674$, $p=0.01$) with the injury percentage. An interesting feature was also observed in some of the X-ray diffraction patterns. A very sharp peak resembling that of an inorganic substance corresponding to an interplanar spacing $d=3.28$ A° was observed for some of the varieties while in others it was absent. The varieties with this characteristic peak show high crystallinity and low injury percentage and hence considered as heat resistant. On the other hand, the varieties in which this peak is absent have low crystallinity and high injury percentage and hence correspond to heat sensitive varieties. The origin of this peak is not yet clearly understood but it has been conclusively shown that the appearance of this peak could be taken as an index—for rapid screening of varieties for their high temperature tolerance.

Introduction

Wheat-growing in India is a gamble in temperature (Howard 1924). The life-cycle of wheat crop in the plains of India covers approximately the period from October-November to March-April during which rainfall is negligible and inconsistent. Towards flowering and thereafter the crop matures under a rapidly increasing temperature when hot dry winds are frequent (Asana, 1976). The vulnerable areas are southern Haryana, Northern Rajasthan and the Malva tract of central India. In addition, during grain development, hot dry winds from Thar desert blow over this area creating sudden increase in the atmospheric temperature. This causes considerable damage to the crop both by way of injuring plant cells as well as creating a condition of atmospheric-induced soil-drought (Asana, 1976). Thus increased heat resistance of wheat crop in irrigated and unirrigated agriculture is of enormous significance. As the high-temperature injury affects the yield (Asana & Williams 1965) it is essential to breed varieties and identify lines which are tolerant to high temperature stress.

Little success is obtained in selection under field testing as it is often complicated by genotype-environmental interactions and impracticable under unpredictable weather conditions. Hence, it is evident that a rapid means of screening resistant strains by simple laboratory technique would help the plant breeders in their hybridization programme.

In two elegant papers Dyson and Lenard (1967, 1968) presented a mathematical proof of the stability of matter and showed that it boils down at the end to an estimate of the binding energy of a single electron in a periodic Coulomb potential. In the light of this it was expected that the crystallinity of tissue materials from different varieties of wheat leaves would be characteristic of their thermal stability. Since X-ray diffractometric technique is one of the most sophisticated and unique techniques it was employed to evaluate X-ray crystallinities of the dried leaves of some wheat varieties to find out the possible varietal differences.

As a general rule it may be postulated that the negentrophy production (Trincher 1965) in plants will serve as an integral index for varietal characterization of plants for their high temperature resistance. Negentropy production in living systems can be measured by microcalorimetric method besides some simple indirect methods. Amongst these the thermal injury evaluated at a specific high temperature through measurement of tissue resistance is found to be quite satisfactory. In the present investigation this parameter was therefore measured for the wheat varieties and their correlations with the X-ray crystallinity values were worked out.

Materials and Methods

A set of 12 wheat varieties (table 1) was taken to evaluate the thermal injury at 40°C as well as the X-ray crystallinity of the dried leaves as these parameters measure the status of thermal tolerance of the varieties.

The varieties were grown on washed autoclaved Yamuna sand (particle size 250-500μ) under identical controlled conditions in a Phytotron. The temperature was maintained at 20$\pm$1°C with a relative humidity of 60%

Table 1 *List of varieties chosen for the present study*

Variety	Species	Some specific characters
C 306	*Triticum aestivum* (Bread wheat)	Drought resistant, native, improved, tall, spring wheat
Kalyansona	,,	Late maturing, mexican dwarf, spring wheat
Sonalika	,,	Early maturing, mexican dwarf, spring wheat
Hira	,,	Dwarf, drought susceptible, spring wheat
Hy–65	,,	Native, improved, tall, spring wheat
Pissi local	,,	Native, Central Indian, Unimproved, tall, spring wheat
Kulu local	,,	Native, north west Himalayan, tall, Semi–winter type
Trope	,,	North American, dwarf, semi–winter type
V 958 odessikaja	,,	Russian, tall, semi–winter type
Bijaga yellow	*T durum* (Macaroni wheat)	Native, improved, tall, spring wheat
Raj 911	,,	Dwarf, spring wheat
Khapli	*T. dicoccum* (Emmer wheat)	Tall, spring wheat

and day length period of 12 hr. Hoagland's solution was uniformly applied to these varieties for providing them with water and nutrients. The seedlings were grown up to a stage where the third leaf from bottom (and second leaf from top) has completely unfurled and reached its physiological maturity (Wicke & Singh 1973). This particular leaf was harvested every time and subjected to analysis.

For the measurement of the tissue conductivity of leaves the technique suggested by Dexter et al. (1932) was suitably modified to suit the present experimental material. A proble was fabricated in the laboratory with platinum-coated sharp steel needles kept at a distance of 1 cm apart. The tissue resistance across the tips of the probe was measured by an Universal Bridge manufactured by Marconi Instrument Ltd, England. The value was obtained at several points in the middle portion of the leaf and was repeated on at least three leaves to get the mean value. The measurement of tissue resistance at room temperature was made on a fresh cut leaf. To obtain the tissue resistance at an elevated temperature a fresh leaf was cut and put in a water-tight packet made out of thin aluminium foil. A slight pressure was applied to the aluminium packet to make uniform contact with the leaf surface without inflicting mechanical damage to it. The packet was immersed in a thermostatically controlled water bath maintained at the desired elevated temperature for 15 min. The packet was then taken out, the leaf removed and the tissue resistance was measured as described above. It was assumed that the thickness of the sampling portion of the leaf was uniform and did not vary significantly between different wheat varieties and species. Since the tissue conductivity is proportional to injury values the percentage of thermal injury (I%) can be evaluated at 40°C (possible maximum temperature during the crop season in some of the vulnerable regions of India) with the help of the following expression

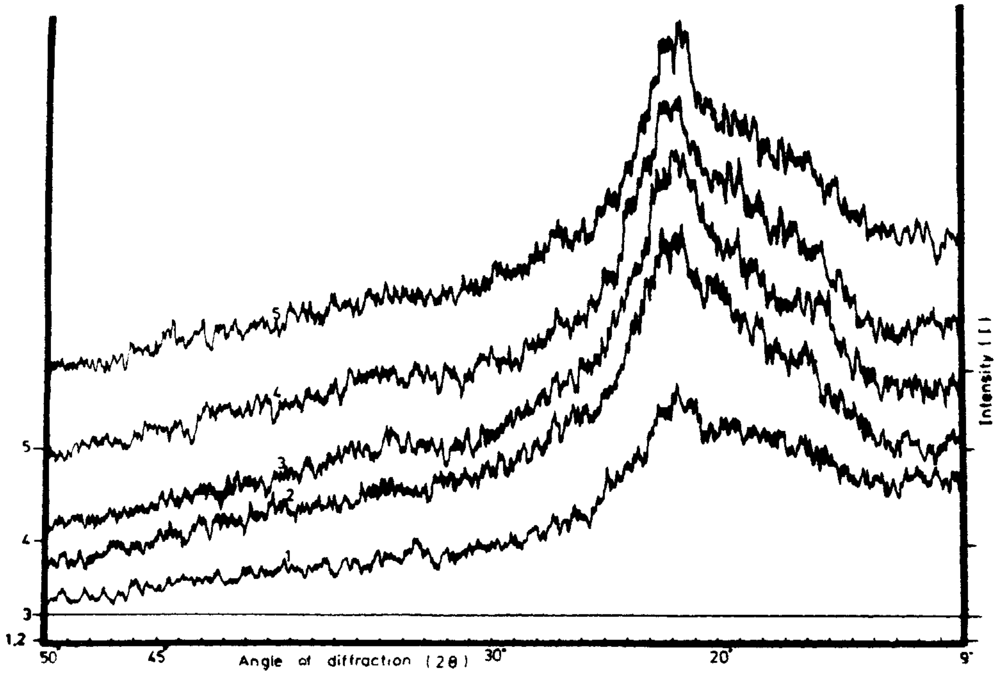
$$I_{40^\circ}\ \% = \frac{(C_{40^\circ}-C_c)}{(C_{max}-C_c)} \times 100 = \frac{(R_c-R_{40^\circ})}{(R_c-R_{max})}\ \frac{R_{max}}{R_{40^\circ}} \times 100 \ldots(1)$$

where C_c is the tissue conductance at room temperature (control), C_{40° is the tissue conductance at 40°C, C_{max} is the tissue conductance at the temperature of maximum killing corresponding to a maximum tissue conductance or minimum tissue resistance and R_c, R_{40° and R_{max} are the corresponding tissue resistances ($R=1/C$) at room temperature, 40°C and maximum killing temperature (60°C in the present case) respectively,

For the measurement of X-ray crystallinity of the dry matter of the leaf, the leaves used for the tissue conductivity measurement at control temperature were oven-dried by keeping them at 105°C for a few minutes followed by drying at 80°C to constant weight (Pandeya et al. 1968) and then ground. The powder specimens were prepared by randomly depositing this powder on glass slides and the X-ray diffractograms of all the 12 samples were taken in the angular range from $2\theta=9°$ to $2\theta=50°$ (figure 1) using Cu Kα radiation at 40 KV, 12mA in conjunction with scintillation counter as detector. Pulse height discrimination was used to enhance the peak to background ratio.

The chemical decrystallization of the powdered leaf samples was carried out following the method of Patil et al. (1965). The sample was immersed for one hour in 71% (w/w) $ZnCl_2$ solution at 35°C. It was followed by thorough washing with ether and air-drying. The air-dried amorphous material was placed in a sample holder and the diffractometer pattern was recorded.

From the X-ray diffractometer patterns (figure 1) the crystallinity percentage was evaluated following Vainshtein (1966) and Meredith and Hearle (1959). A line was


5
4
3
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Angle of diffraction (2θ)
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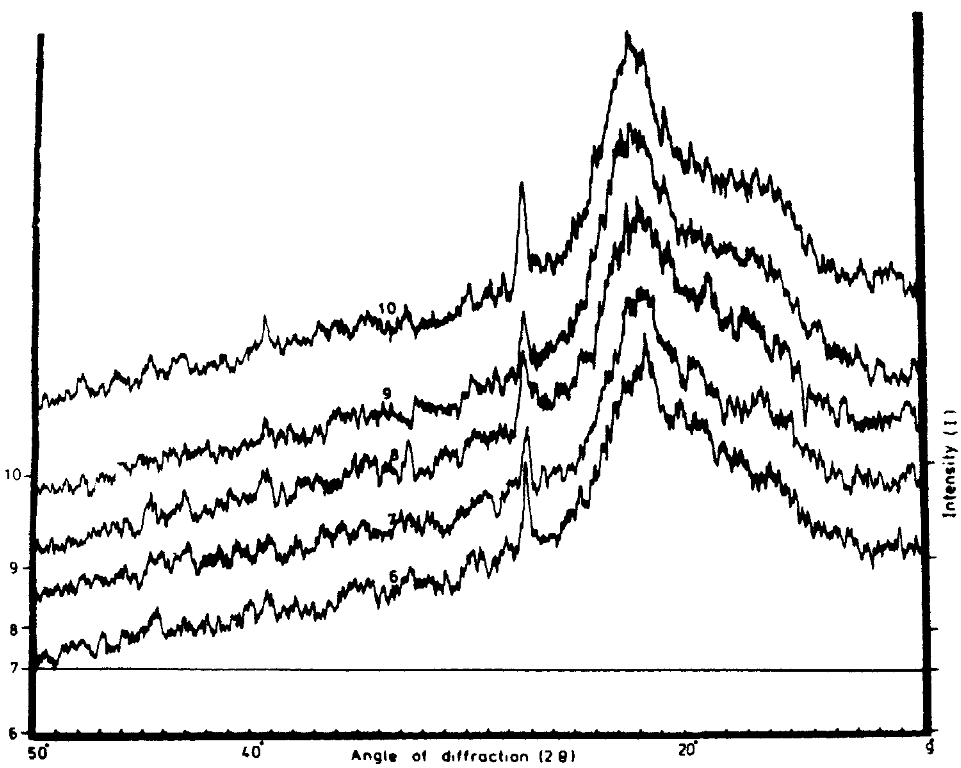
Intensity (I)

A


10
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9
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6
50°
40°
Angle of diffraction (2θ)
20°
9°
Intensity (I)

B

drawn connecting the two extremes of the diffractometer pattern to remove the incoherent background scattering and the area of the pattern above this line was measured using a planimeter. In a similar way the area of the amorphous pattern was measured. As the patterns for different specimens and the amorphous pattern were displaced differentially from the base line, graded versions of the amorphous pattern were made by smoothly matching the amorphous pattern with the sample patterns at the tail end (Meredith & Hearle 1959). These graded areas of the amorphous patterns(A) were subtracted from the total area of the corresponding patterns to get the crystalline areas(C). Now the crystallinity percentage ($C_r\%$) was calculated using the formula.

$$C_r\% = \frac{C}{C+A} \times 100 \quad \ldots \quad \ldots \quad (2)$$

Results and Discussion

The mean resistances at control temperature (R_c), at 40°C (R_{40}°) and at killing temperature (R_{max}) and the injury percentages for all the 12 varieties are given in table 2. By carefully evaluating the variation of conductivity with temperature in the range of room temperature to 90°C it was found that the maximum killing temperature corresponding to the maximum tissue conductivity occurs at 60°C for wheat leaves when the heating time was kept at 15 min. This contradicts the injury evaluation procedure of Sullivan (1972) who arbitrarily took 90°C as the killing temperature for Sorghum. The crystallinity percentages for the varieties, and the total area under the patterns and their respective graded amorphous areas are presented in table 3. The area of the amorphous curve was found to be 21.7 cm^2.

In the tissue-conductivity method the injury values obtained were consistent having a minimum of 1.56% for Kulu local and maximum injury of 13.05% for Hy-65. The semi-winter types namely Kulu local, Trope

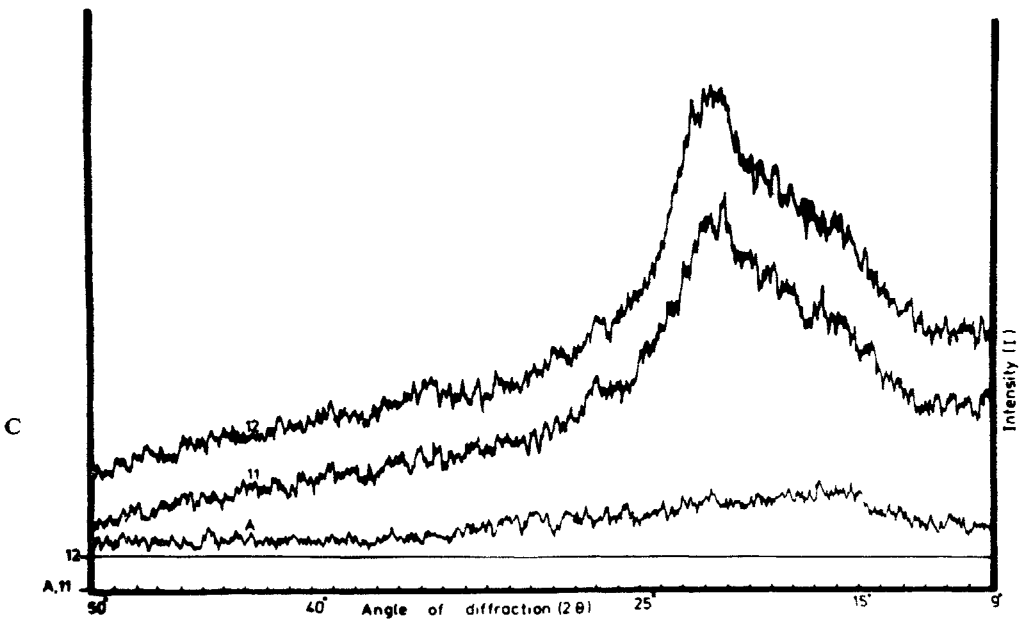

Figure 1. X-ray diffractograms of leaf dry matter
(a) 1: Kalyansona; 2: Hira; 3: Sonalika; 4: Ky-65; 5: Khapli.
(b) 6: V-958 Odessikaja; 7: Raj 911; 8: Pissi local; 9: Kulu local; 10: C-306.
(c) 11: X-ray diffractograms leaf dry matter, Bijaga yellow; 12: Trope A, Amorphous pattern.

Table 2 *Injury percentage by tissue conductivity method*

Variety	Tissue resistance* in 10^6 Ω			Injury percent
	Rc	$R_{40°}$	$R_{60°}$	
C 306	1.174 ± 0.044	1.029 ± 0.053	0.398 ± 0.020	7.23
Kalyansona	1.193 ± 0.070	0.925 ± 0.097	0.342 0.017	11.64
Sonalika	1.065 ± 0.072	0.956 ± 0.040	0.463 ± 0.048	7.51
Hira	1.414 ± 0.032	1.110 ± 0.039	0.363 ± 0.013	9.47
Hy 65	1.134 ± 0.055	0.967 ± 0.088	0.488 ± 0.040	13.05
Pissi local	0.814 ± 0.057	0.721 ± 0.031	0.121 ± 0.010	3.00
Kulu local	0.882 ± 0.059	0.834 ± 0.035	0.188 ± 0.013	1.56
Trope	0.954 ± 0.067	0.840 ± 0.042	0.199 ± 0.018	3.58
V 958 odessikaja	1.413 ± 0.031	1.340 ± 0.038	0.358 ± 0.038	2.61
Bijaga Yellow	0.968 ± 0.028	0.831 ± 0.054	0.326 ± 0.042	8.37
Raj 911	0.989 ± 0.060	0.931 ± 0.061	0.471 ± 0.060	5.67
Khapli	0.817 ± 0.066	0.738 ± 0.063	0.349 ± 0.028	7.97

*Each value is the mean of 18 readings with three replications.

Table 3 *X-ray crystallinity of leaf dry matter*
Area under Amorphous pattern = 21.7 cm^2

Variety	Total area under the pattern (cm^2)	Graded amorphous area (cm^2)	Crystalline area (cm^2)	Crystallinity percentage
C 306*	110.80	47.09	63.71	56.8
Kalyansona	42.40	27.13	15.22	36.0
Sonalika	101.10	47.52	53.58	53.0
Hira	105.70	54.25	51.57	47.8
Hy 65	103.50	54.03	49.47	47.8
Pissi local*	105.70	47.09	58.61	55.5
Kulu local*	118.80	53.17	65.63	53.5
Trope	121.70	51.00	70.80	58.1
V 958 odessikaja*	89.20	33.85	55.35	62.1
Bijaga Yellow	93.00	44.05	48.95	52.6
Raj 911*	88.20	36.67	51.53	58.4
Khapli	94.70	47.96	46.74	49.4

*Varieties which showed a characteristic peak in the pattern.

and V-958 odessikaja which were selected for low temperatures were found to have distinctly lower injury values. This observation gave adequate proof of the hypothesis of Alexandrov (1964) in which he stated that when a plant hardens in autumn it develops low-temperature tolerance and at the same time its high temperature tolerance rises to a maximum paralleling the low-temperature tolerance. By and large it was seen that *durum* species and drought-tolerant *aestivum* wheats have either low or medium injury percentage.

The variety C-306 is well-known for its drought tolerance because of its extensive rooting pattern (Saini 1978). The other tall *aestivum* wheat, Pissi local is a variety from central India where it is adapted to higher temperatures and therefore possesses relatively more heat tolerance. From agronomic trials with limited water, Bhardwaj (1978) has shown that dwarf macaroni wheat (*durum*) performs well and therefore are drought tolerant in nature.

The injury values obtained for Pissi local, C-306, and *durum* wheats were either low or medium (table 2) substantiating the relevance of choosing the injury percentage as an index of intrinsic tolerance.

The X-ray crystallinity values of the dry matter showed a high negative correlation ($r = -0.7674$, $P = 0.01$) with injury percentage showing thereby that higher the crystallinity index the higher the thermal stability of the varieties and hence lower the injury percentage. This observation strongly supported our theoretical assumptions. Apart from the high correlation between X-ray crystallinity and injury values, an interesting observation was made in some of the diffraction patterns. In the diffraction patterns of five varieties namely C-306, Pissi local, Kulu local, V-958 Odessikaja, and Raj 911, a very sharp peak resembling that of an inorganic substance at Bragg angle $2\theta = 27.2°$ with interplaner spacing $d = 3.28$ Å was noticed. These varieties showed high crystallinity values and low injury percentages. The varieties Trope and Bijaga Yellow had slight indications of the peak and were also hardy varieties for heat injury. These seven varieties comprise semi-winter, durum and drought tolerant types of wheat. In general, these varieties could be grouped into two types. One showing a peak or an indication of it at Bragg angle $2\theta = 27.2°$ with crystallinity values exceeding 50% and low injury percentage. Such varieties were considered as heat tolerant. The others which did not show any characteristic peak at Bragg angle $2\theta = 27.2°$ were heat sensitive varieties which in general have crystallinity values less than 50% and high injury percentage. The varieties found in this category were Kalyansona, Sonalika, Hira, Hy-65 and Khapli. It was found that cell-water in wheat leaves undergoes a phase transition at a critical temperature of 60°C.

At present the origin of this above mentioned diffraction peak is not clearly understood. But it was conclusive that the appearance and the non-appearance of this peak could be taken as an index for rapid screening of varieties for their high temperature-tolerance.

Acknowledgement

The authors are grateful to Dr S. Nagarajan, Wheat Pathologist for supplying the seeds of wheat varieties to Dr B. R. Murthy, Project Director, Nuclear Research Laboratory for providing the Phytotron facilities and to Mr Y. V. Subba Rao for making tissue conductivity probe. Thanks are also due to Dr V. G. Bhide, Deputy Director, National Physical Laboratory for his helpful cooperation in the present work.

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