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Screening of wheat varieties for use as donors for production of near isogenic lines with different seed protein alleles

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Abstract

Sequential extraction of water-soluble albumins, salt-soluble globulins (triticin), alcohol-soluble gliadins and acid-soluble glutenins, the four major classes of wheat seed storage proteins, was done from 31 wheat cultivars. SDS-PAGE of the protein fractions showed high variation in the banding pattern. Varieties with different alleles in comparison to the recurrent parent HD 2329 were chosen for all the four fractions of seed storage proteins. A total of 12 cultivars were finally chosen to make NILs for forty different seed storage protein alleles in HD 2329.

Key words: Wheat, seed storage proteins, allelic variation, SDS-PAGE, NILs

Introduction

Wheat flour has the unique ability to form elastic dough when mixed with water and retain gas during fermentation or by chemical leavening to give a leavened product. This characteristic allows more number of varied end products to be made from wheat flour than any other cereal flour. The end-use quality of wheat flour is affected by physical and chemical properties of the endosperm [l]. As compared to carbohydrates and lipids, seed storage proteins are more important factors affecting wheat quality. Water-soluble albumins, saline-soluble globulins, alcohol-soluble gliadins and acid-soluble glutenins are the four major fractions of wheat seed storage proteins, which are synthesized by genes at fifteen major loci [2]. Each locus has multiple alleles giving a possibility of over a million combinations. Each allele, individually, exerts an effect on the rehological properties of wheat flour. Numerous studies have been done for correlating allelic variation to dough properties using different approaches including (i) screening of varieties for allelic composition present and dough properties of flour from these varieties [3], (ii) fractionation and reconstitution studies [4], (iii) use of recombinant inbred lines [5], (iv) genetic engineering [6] and (v) genetic reconstitution using near isogenic lines [7]. To study the relative effect of each allele on end-use quality, NILs having a common genetic background but differing in only one allele of a protein at a time are ideal material as conclusions drawn from such lines are not affected by chemical modification or by pedigree related associations. Further, the different NILs can then be intercrossed to produce any protein combination in a common genetic background. The present study was undertaken to identify potential wheat varieties for developing NILs with different seed storage protein alleles. The variety HD 2329 was chosen as the recurrent parent as it gives dough with medium quality.

Materials and methods

Seeds of 31 wheat varieties were obtained from G. B. Pant University of Agriculture and Technology, Pantnagar; CFTRI, Mysore; DWR, Karnal; Dr. K. W. Shepherd, Adelaide, Australia and Dr. D'Lafiandra, Viterbo, Italy. The genetic stocks were maintained by growing in the field at Pantnagar. Sequential extraction of wheat seed storage protein fractions - albumins, triticin, gliadins and glutenins from a single wheat kernel was done according to Shailaja *et al.* [8].

SDS-PAGE: Different protein fractions were mixed with equal volume of sample buffer [2% SDS, 20% (w/v) glycerol, 1% tris-base, 2mg bromophenol blue, pH 8.0]. Except albumins, all seed storage proteins were incubated at 60°C for 10-15 min for SDS-complexing just before loading in the gel. Albumins (30µl), glutenins (25µl), gliadins (20µl) were loaded on a 10% acrylamide gels with l.5% cross-linking. The gel was made according to the method of Sreeramalu [10]. The amount of triticin fraction loaded per well was 40 µl and the gel prepared was as given by Shailaja *et al.* [8]. Electrophoresis was performed at constant current of 40mA for 2.5-3hrs. The triticin gels, however, were run for a longer time (3.5 hrs) permitting resolution of high molecular weight protein subunits. After electrophoresis gels were stained in coomassie brilliant blue solution [6%(w/v) trichloroacetic acid, 18%

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methanol, 6% glacial acetic acid and 0.025% coomassle brilliant blue R250] overnight and destained in 3% NaCl solution [9].

Results and discussion

The varieties studied showed high level of variation in SDS-PAGE for each protein fraction. In glutenin more polymorphism was visible among low molecular weight subunits than for high molecular weight subunits (Fig. 1). As is evident from in Fig. 1, UP 301 (Lane 15) and UP 2121 (Lane 16) show band 1 at the *Glu-A1* locus which has band 2* in HD 2329 and other varieties. K-68 (Lane 12), UP 319 (Lane 18) and Calidad (Lane 4) are similar for the *Glu-A1* locus for band 2*. The *Glu-B1* locus revealed extensive polymorphism while for *Glu-D1* locus, variation was comparatively less. Immense variation was seen for LMW glutenin subunits. For the *Glu-A3* locus, mobility of band in Kalyansona was higher than HD 2329 (Fig. 1, Lane 9 & 1). Band present in HD 2329 at *Glu-B3* locus was actually absent in HUW 55 (Lane 11). For the *Glu-D3* locus, a band was present in K-68 (Lane 12) unlike HD 2329. Of the varieties screened, nine donors were identified with alleles differing from each other and the recurrent parent at the corresponding loci.

Variation among γ-gliadins and at the *Gli-B1* locus was prominent as seen in the SDS-PAGE for gliadins (Fig. 2). As is clearly visible, Bodallin, Aroona Sr. 22, K-68 and PBW-175 (Lanes 3, 6, 12 and 14) have the same banding pattern at the *Gli-B1* locus, while UP 2121 and UP 319 (Lane 16, 18) have a different one. In WH147, *Gli-D1*, b is present as an extra band below the single band at the same locus in HD 2329 (Fig 2. Lanes 8 & 1). More variation is visible among LMW gliadins. Eight donor varieties were chosen amongst those analyzed for making NILs with different gliadin alleles. On studying the SDS-PAGE for albumins (Fig. 3) variation among polypeptides was visible throughout the length of the gel. Eight varieties were chosen as donors for making NILs with different albumin polypeptides.

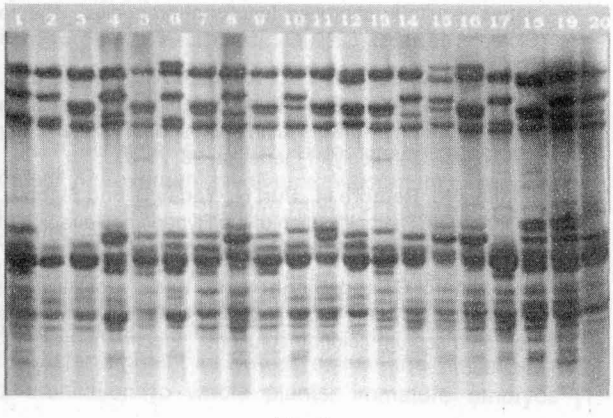

Fig. 1

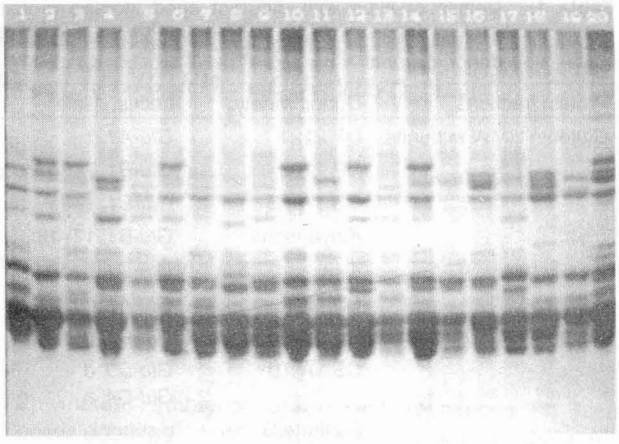

Fig. 2

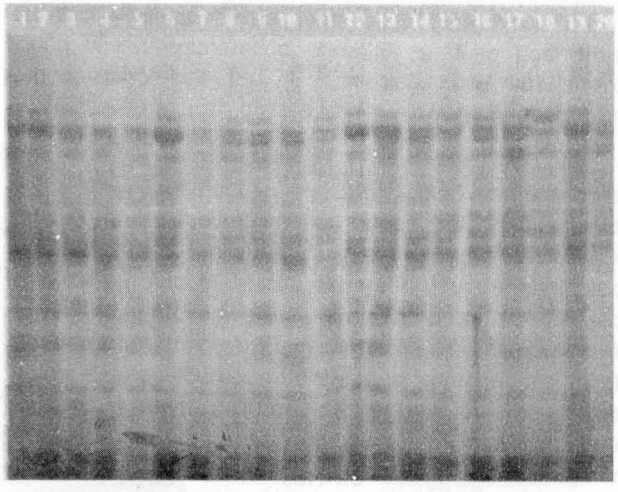

Fig. 3

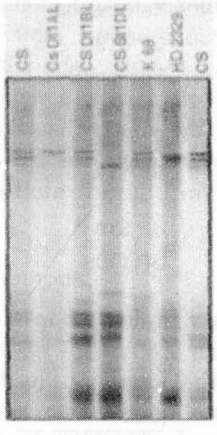

Fig. 4

Figs. 1-4. SDS-PAGE of seed storage protein fractions (1) glutenin, (2) gliadins, (3) albumins and (4) triticin extracted from different wheat cultivars in Lane 1-20. Lanes: (1) HD 2329, (2) Bungulla, (3) Bodallin, (4) Calidad, (5) Millewa, (6) Aroona Sr. 22, (7) WL 711, (8) WH 147, (9) Kalyansona, (IO) Sunkota B, (11) HUW 55, (12) K-68, (13) PBW 65, (14) PBW 175, (15) UP 301, (16) UP 2121, (17) UP 115, (18) UP 319, (19) UP 262, (20) Chinese spring.

Table 1. Details of NILs with different seed storage protein alleles being developed from varieties chosen as donor parents

Protein fractions	Donor variety	Locus, allele
Glutenin HMW subunits	UP 2121	*Glu-A1*, 1
	UP 1109	*Glu-B1*, 7+8
	CS 1A-1B	*Glu-B1*, 7+8*[1]
	Kalyansona	*Glu-B1*, 17+18
	K-68	*Glu-D1*, 5+10
	CS 1BL	*Glu-A1*, null
Glutenin LMW subunits	CS 1BL	*Glu-D3*, d
	CS 1A-1B	*Glu-D3*, d *Glu-D4*, a
	Sunkota B	c-subunit, sb
	Kalyansona	*Glu-A3*, c c-subunit, ks ω-type, ks
	UP 319	*Glu-A3*, d
	UP 1109	*Glu-A3*, d[2]
	UP 115	*Glu-A3*, e
	HUW 55	*Glu-B3*, d *Glu-D3*, e
	K-68	*Glu-D5*, b
Gliadins	WH 147	*Gli-A1*, g *Gli-D1*, b γ-*gli*, mb
	UP 115	*Gli-A1*, h γ-*gli*, md
	CS 1A-1B	*Gli-B1*, a
	UP 2121	*Gli-B1*, b
	UP 1109	*Gli-B1*, c* γ-*gli*, ma
	Kalyansona	*Gli-B1*, d
	UP 319	γ-*gli*, mc
	Sunkota B	γ-*gli*, me
Albumins	UP 301	ma mb
	UP 115	mc md
	UP 319	me
	UP 1109	mf mc[3]
	K-68	mg mh
Triticin	K-68	*Tri-A1*, a
	K-68	*Tri-D1*, a

[1]Band 7 from Chines Spring was of lighter intensity as in comparison to that from UP 1109.
[2]Same allele but different donor
[3]Same allele but different donor for protein fraction albumin, allele mc

The triplet bands for triticin were clearly resolved in the SDS-PAGE using the method developed by Shailaja *et a.* [11] as shown in Fig 4. For triticin, we focused only on the triplet bands occurring at the cathodal half of the gel. There are only two functional gene products for triticin, which are coded by genes at the *Tri-A1* and *Tri-D1* loci and each locus has two alleles only. K-68 was the only donor chosen for making NILs in wheat with different triticin alleles as it had both the different allelic forms in its genetic constitution as compared to the recurrent parent HD 2329. The total number of varieties chosen for analysis of different seed storage proteins was 12.

The 12 donor varieties and alleles chosen for making NILs in wheat with different seed storage protein alleles are given in Table 1.

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