Prevalence of Puroindoline Genes and Their Impact on Quality Traits in A Diverse Germplasm of Wheat Genotypes

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ABSTRACT

Grain hardness is an imperative attribute that determines the end-use quality of wheat. Variation in grain hardness is usually controlled by Puroindoline (pin-a and pin-b) genes located on the 5D chromosome. The study was aimed to reveal different mutations in Puroindoline genes utilizing the STS-marker approach and their association with important quality attributes in 100 hexaploid wheat genotypes (96 from Pakistan and 4 from CIMMYT). Overall, seven puroindoline genes were identified. Among them Pina-d1b(null) (85%) was most common while Pinb-d1i (1%) and Pinb-d1ab (1%) were most rare gene. Out of 100 genotypes, 97 had hard texture either with single or double mutant pin-genes, while three had a soft texture with wild type (Pina-d1a/Pinb-d1a) pin-genes. All four quality attributes revealed a vast deviation among germplasm, while their correlation analysis revealed the highest association (r=0.71) between thousand-grain weight and protein content. In addition, three out of four quality traits, i.e. thousand-grain weight, SDS-sedimentation value and protein content, showed the highest mean values for double mutant (Pina-d1b/Pinb-d1b) followed by single mutant, i.e. Pina-d1b. The present study facilitates breeders for varietal selection (hard or soft) according to end-use quality and offers valuable information for improving wheat quality.
an efficient and most reliable substitute for many conventional techniques. It helps amplify the target genes and makes advancements in the research study of specific genes for desirable traits (Garibyan & Avashia, 2013). Different studies detected mutation in puroindoline (Pina and Pinb) genes using other techniques, i.e. STS-allele-specific markers, cleaved amplified polymorphic sequences (CAPS), SNP assays and pyrosequencing (Maryami, Azimi, Guzman, Dreisigacker, & Najafian, 2020; Przyborowski, Gasparis, Kala, Orczyk, & Nadolska-Orczyk, 2020). A genomic study of puroindoline alleles revealed that the Pina gene could not be expressed in hard textured wheat as exhibiting mutation in the Pina gene. At the same time, the ratio of Pina/Pinb expression was usually lesser in the complex textured genotypes. Mutations in the coding sequence of Pin proteins could also be linked with variations in puroindoline (Pin) expression and other features (Nirmal, Furtado, Wrigley, & Henry, 2016).

The impact of puroindoline genes on hardness and mealiness was elaborated by Presinszka et al. (2016). They reported that different wheat genotypes exhibited a variety of grain colours such as: blue aleurone, yellow endosperm, white and purple pericarp and grain hardness varied from 15.10 to 26.87 N/sample having various mutations within puroindolines (Pina and Pinb) such as Pinb-D1c, Pinb-D1b, Pina-D1b and Pinb-D1d. Different mutations in puroindoline genes (Pina and Pinb) found in Pinb, such as: Pinb-(D1p, D1c, D1b and D1e), are responsible for complex phenotype and existed in the form of different combinations such as: PinaD1b/PinbD1a, PinaD1a/PinaD1p, PinaD1a/PinbD1e and PinaD1a/PinaD1c, PinaD1a/PinbD1b. Genetic modification of wheat with specific Puroindoline genes could be helpful for the development of new and improved plants with desirable properties (Qamar et al., 2014). Association of allelic variation of Puroindoline b-B2 and D1 showed a non-significant difference for grain hardness (SKCS) among genotypes with Pinb (B2v2 & B2v3) alleles surrounded by the 2 Puroindoline-D1 haplotypes. As a result, the PIN b-B2 gene was highly associated with soft textured wheat than harder one (Chen et al., 2010).

Hard wheat is suitable for bread making as it contains high quality of protein, high particles size, as well as high damaged starch (Pauly, Pareyt, Fierens, & Delcour, 2013). Puroindoline genes are directly related to grain hardness and milling quality to depict the end-use purpose of specific wheat genotypes (Wang et al., 2018). The fluctuations in quality traits depend on mutations in Pina & Pinb genes as the soft texture genotypes with wild form (Pina-d1a/ Pinb-d1a) had lower values of quality traits i.e. SDS-sedimentation value, protein content, particles size index, and damaged starch, than the harder ones. The highest grain hardness among complex genotypes with Pina-d1b (null) and Pina-d1k/Pinb-d1q had the highest grain hardness due to lack of Pina & Pinb expressions (Takata, Ikeda, Yanaka, & Ishikawa, 2008).

The valuable information about grain hardness and grain milling quality would be needed to determine the end-use purpose of commercial varieties. Therefore current study characterized diverse wheat germplasm based on essential quality parameters that play a critical role in wheat quality. However, molecular identification of puroindoline genes (grain hardness) and their impact on vital quality parameters was also determined. The proper interpretations from the current study would be advantageous for bakers and breeders to select the superior varieties according to their specific goals. In addition, the deleterious Pin-genes can also be replaced by introgression of advantageous Pin-genes to obtain related to excellent wheat quality.

MATERIALS AND METHODS

Plant Material and Phenotyping

Hundred wheat varieties (96 from Pakistan and 4 from CIMMYT) were cultivated in Research Farm of PMAS-Arid Agriculture University, Rawalpindi during the two Rabi seasons (2015-2016 and 2016-2017) under rain-fed conditions.

Thousand-grain weight was calculated by counting a thousand grains from each variety using seed counter and then weighed in grams (Awan, Ahmad, Ali, Ahmed, & Rao, 2015). SDS-sedimentation value was determined by measuring the volume of sediments as the consequence of applying lactic acid on the mixture of water-flour according to AACC (2000; Method 56-63.01). It was measured in millilitres (ml). Protein was determined according to Dumas combustion method. Combustion was done at high temperature in a protein analyzer (Leco FP-528). Protein was...
estimated by calculating the amount of nitrogen escaped during combustion according to AACC (2000; Method 46-30. It was measured in g/100 g. The total amount of inorganic matter in flour is referred to as ash content. Each flour sample (3 g) was weighed and put in to muffle furnace at 590°C. The ash content was recorded according to AACC (2000; Method 08-01.01.

Genotyping

DNA Extraction

Four seeds of each variety were grown to obtain seedlings in Western Wheat Quality Laboratory’s nursery in Washington State, USA. After two weeks, the seedlings were kept in a speed vac. SVC 200 to dry for two days. The leaves were disrupted and homogenized with the help of steel beads shaken at high speed under a tissuelyser system to grind samples. Genomic DNA was extracted through DNeasy (Quiagen) 96 Plant Kit, highly efficient for DNA extraction of multiple samples (96-192) all together (Ibba, Kiszonas, Guzmán, & Morris, 2017).

Sequence Tagged Site (STS) Allele-specific Marker

Already reported Sequence Tagged Site (STS) allele-specific markers were utilized for genetic dissection of puroindoline genes at Pina-d1 and Pinb-d1 loci (Table 1).

| Marker name | Sequence | Amplicon size (bp) | Reference |
|-------------|----------|--------------------|-----------|
| PinanullF   | AATACCACATGGTTCTAGACTG GCAATACAAAGGACCTCTAGATT | 776 | Chen et al., 2010 |
| PinanullR   | AGTACACGGAACATCATATATCTCAA ACCACTKTATTTGTCACACGAG | 699 | Morris et al., 2011 |
| Pinaf1f2    | TCAACATTCGTGCATCATCA CTTCATGCTCACAGCCGCT | 436 | Chen, Zhang, Xia, Dong, & Cui, 2012 |
| Pina-D1r    | ATGAAGACCTTATTCCTCCTA CTCATGCTCACAGCCGCT | 250 | Giroux & Morris, 1997 |
| Pinbflr3    | ATTAGAACCCTTTATCTCTCA CTCATGCTCACAGCCGCT | 250 | Giroux & Morris, 1997 |
| pinbfseqf1  | TTGTATACCAACTTAATTTG sequencing | 745 | Morris et al., 2011 |
RESULTS AND DISCUSSION

Phenotyping

The descriptive statistics of two years (2015-2016 and 2016-17) pooled data showed different levels of variation for important quality attributes among 100 genotypes (Table 2). The highest variation was exhibited by thousand-grain weight, followed by SDS-sedimentation value and protein content. Thousand-grain weight ranged from 24.24 g to 47.79 g, SDS-sedimentation value ranged from 15.44 ml to 29.49 ml, while protein content varied from 9.0 g/100 g to 13.88 g/100 g. However, ash content exhibited the lowest variation as its range was 1.13 g/100 g to 1.81 g/100 g. Earlier studies revealed that the mean values of thousand-grain weight varied from 27.32 g to 56.14 g (Awan, Ahmad, Ali, Ahmed, & Rao, 2015). SDS-sedimentation ranged from 15.0 ml to 38.0 ml (Ahmed & Fayyaz-ul-Hassan, 2015) in Pakistani wheat germplasm sown in different locations. The mean values of protein content are strongly consistent with those reported by previous studies (Tehseen, Anjum, Pasha, Khan, & Saeed, 2014), revealed the content of protein varied with the range of 9.43 to13.96% in numerous Pakistani wheat varieties. These results are in line with those of the current study. Ferrari, Clerici, & Chang (2014) indicated the broad range of protein content was associated with broader application of end-use products. The previous studies reported low variation of ash content (Khan, Memon, Deverajani, & Baloch, 2015), who revealed the ash content in various Pakistani wheat genotypes diverged from 1.34 to 1.81 g/100 g. Ash content is an important quality attribute that determines the purity of flour and the colour of the end product (Tehseen, Anjum, Pasha, Khan, & Saeed, 2014).

Correlation among Quality Traits

The correlation study depicted sufficient association among different quality traits (Table 3). A highly significant and positive relationship was found between thousand-grain weight, SDS-sedimentation value, and protein content, while ash content could not show association with any studied trait. The highest and positive correlation was exhibited by protein content for the thousand-grain weight (r=0.71) and sedimentation value (r=0.70). Protein content was positively and significantly associated with thousand-grain weight and sedimentation value (Drikvand, Bihimta, Najafian, & Ebrahimi, 2013). Khan, Memon, Deverajani, & Baloch (2015) determined that the thousand-grain weight was positively correlated with grain protein (r=0.6). The protein content is directly associated with thousand-grain weight. These findings are in close agreement with the current study. However, Al-Saleh & Brennan (2012) reported a significant and positive relationship between protein percentage and ash content, contrary to this study. The different consequences for ash content were due to various genetic make of varieties, agronomical treatments, and agro-climatic conditions.

The current study suggested that the higher values of thousand-grain weight, SDS-sedimentation and protein concentration are considered good indicators of a wheat quality suitable for bread making.

Table 2. Descriptive statistics of different quality traits among diverse wheat genotypes

| Traits                        | Minimum | Maximum | Mean  | Std. Deviation | Variance |
|-------------------------------|---------|---------|-------|----------------|----------|
| Thousand grain weight (g)     | 24.24   | 47.79   | 39.50 | 5.34           | 28.54    |
| SDS-sedimentation value (ml)  | 15.44   | 29.49   | 24.42 | 3.62           | 13.08    |
| Protein content (g/100g)      | 9.00    | 13.88   | 11.71 | 1.41           | 1.99     |
| Ash content (g/100g)          | 1.13    | 1.81    | 1.46  | 0.18           | 0.03     |

Table 3. Pearson’s correlation coefficients (r) between different quality traits

|                         | Thousand grain weight | SDS-sedimentation value | Protein content | Ash content |
|-------------------------|-----------------------|-------------------------|-----------------|-------------|
| Thousand grain weight   | 1                     | 0.61**                  | 0.71**          | 0.08        |
| SDS-sedimentation value | 0.61**                | 1                       | 0.70**          | -0.12       |
| Protein content         | 0.71**                | 0.70**                  | 1               | 0.03        |
| Ash content             | -0.08                 | -0.12                   | 0.03            | 1           |

Remarks: ** Highly significant \( \leq 0.001 \)
Prevalence of Puroindoline Genes and Their Impact on Quality Traits

Grain hardness profile of wheat genotypes was analyzed to detect different mutations in puroindoline "a" & puroindoline "b" genes at Ha locus (Table 4, Fig. 1). Seven puroindoline genes were identified, which occurred in different combinations. At the pina-d1 locus, the allelic variant Pina-d1b (null) was most commonly presented in 85 genotypes, while at the pinb-d1 locus, the allelic variants i.e., Pinb-d1i (1%) and Pinb-d1ab (1%) were the rarest genes. Two different combinations of pin-genes were double mutants Pina-d1b/Pinb-d1i (2%) and Pina-d1b/Pinb-d1b. Two genotypes harboured Pina-d1a/Pinb-d1b combination while Pina-d1a/Pinb-d1a was wild type combination occurred in three genotypes exhibited soft texture. Out of 100 genotypes, 97 had hard texture either with single or double mutant pin-genes.

Table 4. Relationship of Puroindoline genes with quality traits and their distributions in wheat genotypes

| Pin-genes         | No. of genotypes | Thousand grain weight | SDS-sedimentation value | Protein content | ASH |
|-------------------|------------------|-----------------------|-------------------------|----------------|-----|
| Pina-d1b(null)    | 85               | 40.33                 | 24.84                   | 11.82          | 1.42|
| Pina-d1a/Pinb-d1b| 5                | 36.00                 | 24.60                   | 11.46          | 1.60|
| Pina-d1a/Pinb-d1a| 3                | 25.63                 | 16.50                   | 9.25           | 1.77|
| Pina-d1b/Pinb-d1i| 2                | 30.82                 | 20.55                   | 10.36          | 1.69|
| Pina-d1u/Pinb-d1a| 2                | 32.84                 | 25.31                   | 11.47          | 1.62|
| Pina-d1b/Pinb-d1b| 1                | 46.67                 | 29.49                   | 12.19          | 1.33|
| Pina-d1a/Pin b-d1ab| 1            | 28.77                 | 20.04                   | 11.35          | 1.69|
| Pina-d1a/Pinb-d1i| 1                | 35.71                 | 23.55                   | 10.84          | 1.76|

Fig. 1. Prevalence of different puroindoline genes among diverse genotypes
In addition, these allelic variants of hardness showed association with quality traits (Table 4 and Fig. 2, Fig. 3, Fig. 4, and Fig. 5). Three out of four quality traits i.e. thousand-grain weight, SDS-sedimentation value and protein content, showed the highest mean values for double mutant (Pina-d1b/Pinb-d1b) and followed by single mutant i.e. Pina-d1b (null). The genotypes with Pina-d1b/ Pinb-d1b double mutants showed the highest mean values for the thousand-grain weight (46.67 g), SDS-sedimentation value (29.49 ml) and protein content (12.19 g/100 g). The genotypes with Pinb-d1b (null) showed mean values for the thousand-grain weight (40.33 g), SDS-sedimentation value (24.84 ml) and protein content (11.83 g/100 g). The negative impact was revealed for ash content by double mutant 1.33 g/100 g and followed by Pina-d1b (null) 1.42 g/100 g. However, wild type combination Pina-d1a/Pinb-d1a exhibited the lowest values for thousand-grain weight (25.63), SDS-sedimentation value (16.50 ml) and protein content (9.25 g/100 g) while highest values for ash content (1.77 g/100 g).

Fig. 2. Distributions of Puroindoline genes and their association with thousand grain weight
Fig. 3. Distributions of Puroindoline genes and their association with SDS-sedimentation value
Fig. 4. Distributions of Puroindoline genes and their association with protein content
The results are in line with the study of Ribeiro et al. (2017), who found that SDS-value and protein content were highly associated with a mutation in Pin-genes while the genotypes with wild-type purindoline were lowered in quality traits. A similar study was conducted by Takata, Ikeda, Yanaka, & Ishikawa (2008), who detected higher mean values of SDS-sedimentation value, protein content, particles size index, and damaged starch in complex genotypes than the softer ones. Three genotypes i.e. Punjab-85, Raskoh and ZA-77, had the wild type combination of Pina-d1a/Pinb-d1a associated with the soft texture of the grain. The flour quality and wheat processing could be improved more easily in super soft texture wheat (Aoun, Carter, Ward, & Morris, 2021).

Rare mutation (Pinb-D1ab) at pinb locus exhibited by only one genotype, i.e. Local white had hard grain texture. The mutation Pinb-d1i was exhibited by three genotypes, i.e. Pakistan-13,
Hoosam-3 and NR-520. Pinb-d1b mutation was shown by genotypes, i.e. Sarhad-82, FSD-85, Khyber-87, Dera-98 and Iqbal-2000. Wild-type purindoline (Pina-d1a and Pinb-d1a) alleles are determinants of soft grain texture, and any mutation can lead to variations (hard texture) in the caryopsis of grain (Ma et al., 2017).

Hard wheat flour is most suitable for bread and bun making associated with the highest SDS-sedimentation value, protein. In contrast, soft wheat flour lowered in SDS-sedimentation value, protein usually utilized for cookies, crackers, biscuits, cakes, noodles, pastries etc. (Pauly, Pareyt, Fieren, & Delcour, 2013). Giroux & Morris (1997) revealed that deletion of the gene from Pina-d1 caused the variation from Pina/d1a to Pina/d1b. Pina/d1b (pina-null) is the most prevalent mutation which included the representative commercial varieties as; Augab-2000, Seher-06, Zamidar-80, Zarghoon-79, Mexipak-65, Lu-26, Galxy-13. Ikeda, Cong, Suzuki, & Takata (2010) discovered the most frequent mutation (gene deletion) in purindoline ‘a’ Pin-a null (Pina-d1b), broadly dispensed in common wheat varieties. Pina/d1b or Pin-a null is the worldwide most prevalent mutation and found with hardest texture on the basis molecular as well as physical basis (Nucia, Okoń, Tomczyńska-Mleko, & Nawrocka, 2021).

Ma, Zhang, Xia, Morris, & He (2009) reported that the Pina-d1b/Pinb-d1a mutation is responsible for the hardest grain texture. Vee/Nac is hardest wheat variety exhibited Pina-d1b/Pinb-d1a mutation. Five other point mutations in Pinb-d1 were found in germplasm from different geographical origins (Bhave & Morris, 2008). Four different mutations i.e. Pina-d1b (null), Pina-d1b, Pinb-d1ab and Pinb-d1i were found in current studies. Similar mutations were found in previous studies, as Mohammad, Mehrzaz, Izadi-Darbandi, & Najafian (2013) found Pina-d1a/Pinb-d1a the most frequent combination of purindoline genes responsible for a soft texture. Mu et al. (2008) also found a double mutant genotype (named Maroon) with the combination of pina-d1b/pinb-D1d in the CIMMYT wheat nursery. High diversity of purindoline genes was found in wild species i.e., wild einkorn. The introgression of diverse Pin-genes from wild species to common wheat could be useful for end-use quality (Huertas-García, Castellano, Guzmán, & Alvarez, 2021).

CONCLUSION

Genetic dissection of purindolines (Pina and Pinb) genes provides complete information related to the grain texture of wheat and their association with important quality traits. This knowledge may help to determine the end-use quality of specific wheat varieties. The evaluated germplasm mainly comprises complex wheat genotypes considered appropriate for making bread, chapatti, Naan, Tortilla, etc. However, three soft textured genotypes are deemed suitable for making biscuits, cookies, crackers, cakes, etc. Moreover, grain texture profiling also provides an essential prospect for exploiting more genetic variability to improve grain texture properties in future breeding programs.

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