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Research Paper

Expression of seed storage proteins responsible for maintaining kernel traits and wheat flour quality in common wheat under heat stress conditions

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Heat stress during grain filling has been documented to decrease wheat grain yield and quality in arid regions worldwide. We studied the effect of heat stress on wheat flour quality in heat tolerant cultivars to define the effects of heat stress on flour quality and to identify germplasm combining traits for heat tolerance and good flour quality. We studied the kernel phenotypic traits, the expression of seed storage proteins (SSPs), and the resulting flour quality under heat and normal conditions. Under heat stress, all cultivars yielded narrow-shaped seeds, and increased protein contents as compared to the control plants grown under normal conditions. The specific sedimentation values used to estimate the gluten quality varied between cultivars. We identified cultivars that could maintain good flour quality under heat stress conditions: 'Imam', which possessed the *Glu-D1d* allele responsible for the suitable bread-making; 'Bohaine', which displayed high expression level of SSPs; and 'Condor', which possessed slight variations in the ratio of each SSP under heat stress conditions. Combining the desirable traits from these cultivars could yield a wheat cultivar with heat tolerance and good flour quality.

Key Words: *Triticum aestivum* L., heat stress, kernel traits, wheat flour quality, seed storage protein.

Introduction

Global wheat production is estimated to decrease by 6% for each 1°C increase in the global mean temperature (Asseng *et al.* 2015). High temperature affects wheat in different ways including poor germination and plant establishment, reduced photosynthesis, leaf senescence, decreased pollen viability, and consequently production of less grains with smaller grain size (Asseng *et al.* 2011, Ugarte *et al.* 2007). Heat stress during the grain-filling stage in wheat significantly modifies kernel traits, grain protein content and composition, adversely affecting the overall yield and quality. Kernel traits are not only directly related to the grain yield, but also the milling yield that could be increased by optimizing grain shape and size with large and spherical grains being the optimum grain morphology (Evers *et al.* 1990). High temperatures (>30–35°C) during grain filling are reported to have a weakening effect on bread dough strength (Randall and Moss 1990) due to associated changes in the composition of the gluten proteins (Daniel and Triboi 2000) and an increase in the ratio of gliadins to glutenins. Tahir *et al.* (2006) reported that in field-grown genotypes, high temperatures increased gluten strength and decreased dough elasticity.

Grain proteins are primarily composed of seed storage proteins (SSPs), the composition and subsequent functionality of which vary among and within wheat classes. In the general brands on the market, flour made from "hard wheat" is recommended for bread making, as it produces dough with favorable gas-holding properties and highly leavened bread. In contrast, "soft wheat" flour displays inferior gas-holding properties and is generally utilized to make cookies, cakes, and crackers. These functional traits are related to the physical properties of hydrated gliadins and glutenins. Gliadins are viscous and easily stretched, while glutenins are highly elastic and provide resistance to stretching. When combined, these two proteins form gluten, which has viscoelastic properties. The technical properties of wheat flour are therefore directly related to the ratio of gliadins to glutenins in the flour.

Gliadins are divided into four groups of bands (α-, β-, γ-, and ω-gliadins) based on their mobility in acid-polyacrylamide gel electrophoresis (A-PAGE) (Bushuk and Zillman 1978). The γ- and ω-gliadins are encoded by the *Gli-1* loci, designated *Gli-A1*, *Gli-B1*, and *Gli-D1* on the

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short arms of the homoeologous group-1 chromosomes 1A, 1B, and 1D, respectively. The α- and β-gliadins are encoded by the *Gli-2* loci, designated *Gli-A2*, *Gli-B2*, and *Gli-D2* on the short arms of the homoeologous group-6 chromosomes 6A, 6B, and 6D, respectively (Payne *et al.* 1984). Glutenins are composed of high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs). The HMW-GSs are encoded by the *Glu-1* loci, designated *Glu-A1*, *Glu-B1*, and *Glu-D1* on the long arms of the homoeologous group-1 chromosomes 1A, 1B, and 1D, respectively (Payne *et al.* 1980). The LMW-GSs are subdivided into B, C, and D types according to the mobility of their relative isoelectric points and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels by two-dimensional electrophoresis (2-DE) (Jackson *et al.* 1983). The B-type LMW-GSs are typical LMW-GSs and are encoded by the *Glu-3* loci, designated *Glu-A3*, *Glu-B3*, and *Glu-D3* on the short arms of the homoeologous group-1 chromosomes 1A, 1B, and 1D, respectively (Gupta and Shepherd 1993). The *Glu-3* homoeoloci are tightly linked to the corresponding *Gli-1* homoeoloci on the short arms of chromosomes 1A, 1B, and 1D (Singh and Shepherd 1988).

In a comparison of 84 British-grown varieties, Payne *et al.* (1987) demonstrated that the HMW-GS and *Glu-D1d* alleles tended to exhibit the highest *Glu-1* quality score, a metric relating individual HMW-GSs to bread-making quality. The two HMW-GS genes of *Glu-D1d* are *1Dx5* and *1Dy10* (specifying the 1Dx5 and 1Dy10 subunits, respectively). The 1Dx5 subunit harbors an amino acid substitution at position 118, causing the replacement of a serine residue with cysteine at the beginning of the repetitive domain (Anderson *et al.* 1989). The presence of this extra cysteine in 1Dx5 allows linkage via intermolecular disulfide bonds and the formation of larger insoluble polymers. These gluten protein polymer size and complexity in the mature grain and changes during dough formation are important for bread making (Johansson *et al.* 2013). It is therefore frequently associated with doughs exhibiting stronger elasticity and superior end-use qualities for bread making.

The other allele of HMW-GS, *Glu-B1al*, also has a positive effect on bread making. The presence of *Glu-B1al* results in a significant increase in the expression of the 1Bx7 subunit (Marchylo *et al.* 1992). This overexpression results in an increase in dough strength and is considered to be the reason for the positive relationship between the *Glu-B1al* allele and high bread-making quality (Lukow *et al.* 1992). These findings indicate the importance of *Glu-D1d* and *Glu-B1al* and their effects on protein structure and protein expression for bread-making quality. These positive effects on bread-making quality were, however, evaluated in plants grown under normal conditions. The threats of global warming, accelerating climate change and their effects on crops make the detailed understanding of heat stress on flour quality of paramount importance. We evaluated the effect of heat stress on kernel shape, flour quality, and the expression of glutenins and gliadins in heat tolerant wheat cultivars grown under heat stress conditions to identify promising wheat germplasm with both heat tolerance and good end-use quality traits for use in breeding programs.

Materials and Methods

Plant materials and growth conditions

We used the Japanese commercial common wheat cultivar 'Norin 61' and the heat tolerant Sudanese common wheat cultivars 'Imam', 'Condor', 'Tagana', 'Bohaine', and 'VYT11' (Proceedings of the Variety Release Committee, Ministry of Agriculture and Forestry, Sudan). The Sudanese varieties have been intensively tested under the heat stress conditions of Sudan and released officially for the commercial cultivation under these stress conditions (Tadesse *et al.* 2019, Tahir *et al.* 2006, 2020). Twelve seeds from each cultivar were sown in nursery boxes containing nursery soil (SK agri Co. Ltd, Japan) in late October. Twelve seedlings were transferred to a total of six pots, two seedlings in each unglazed No. 6 pot (18 cm diameter) containing 2.2 L organic soil. Each pot was considered a replication. They were kept at a light intensity of 80,000 lux under a 14/10 h day/night photoperiod (22/18°C, 40/50% relative humidity) until heading (60 days on average) using growth chambers at the Arid Land Research Center, Tottori, Japan. At heading, half (three pots) of the 12 plants of each cultivar were transferred to another chamber set at 38/18°C day/night for exposure to heat stress up to maturity, following the methods of Elbashir *et al.* (2017). Briefly the daytime temperature was gradually increased by 4°C hr^{-1} from the nighttime temperature of 14°C until reached the maximum temperature of 38°C, kept there for four hours and then gradually decreased in the same manner to the night temperature. The other half (three pots) of the 12 plants of each cultivar were allowed to continue to grow under the same conditions (normal) as before heading. In each chamber (normal and heat stress) the experiment was arranged in a completely randomized design with three replications.

Phenotypic evaluation of the kernel

We evaluated the kernel shape traits and size traits as follows. One hundred fully developed kernels were visually selected from each cultivar under each condition. Digital pictures of the kernels were taken from both horizontal and vertical aspects, both using 50 kernels; the latter were obtained by cutting one distal end of the kernel so that it could be positioned upright. Images were analyzed using ImageJ software (National Institutes of Health, USA, imagej.nih.gov/ij/), according to Breseghello and Sorrells (2007). Approximately 250 kernels were randomly selected and +weighed to determine the average kernel weight (mg, KW) for each cultivar under each condition. The data were

statistically analyzed using JMP software ver. 12.0.1 (SAS Institute).

Evaluation of flour quality

To evaluate the flour quality for each of the wheat cultivars, 5 g of seeds from each cultivar under each condition were ground in a UDY cyclone sample mill (UDY Corp., Fort Collins, CO, USA) fitted with 1-mm screens. The protein content (%, PC) of the ground seeds was then measured by near-infrared spectroscopy (Kett, model KJT-270, NIR composition analyzer). The SDS sedimentation volume (SDS-SV) is highly correlated with bread loaf volume and is a reflection of gluten quantity and quality (Axford *et al.* 1979); we measured the SDS-SV in 1 g of flour, according to the methods of Takata *et al.* (1999). For an index of gluten quality, specific sedimentation values (SSVs) were calculated by dividing the SDS-SV by the percentage of PC because the PC of wheat is reported to be highly correlated with the SDS-SV (Moonen *et al.* 1982). The data were statistically analyzed using JMP software ver. 12.0.1 (SAS Institute).

Separation of SSPs by one-dimensional electrophoresis

Three fully developed kernels were selected from each cultivar under each condition. The embryos were removed, and the endosperm was hammer-milled to a fine powder, and then 600 µL of 70% (v/v) aqueous ethanol was added to the powder of the three kernels. The mixture was incubated in a closed microtube for 30 min at 40°C, and centrifuged for 3 min, at 1,800 × g.

For expression analysis of gliadins, the supernatants containing gliadins extracted from the powder of the three kernels were loaded onto a gel for acid-polyacrylamide gel electrophoresis (A-PAGE) according to the methods of Tanaka *et al.* (2003).

For expression analysis of glutenins, the pellets containing glutenins extracted from the powder of the three kernels were suspended in a solution, and then loaded onto a gel for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The methods of Tanaka *et al.* (2003) were followed for this step, but 12% polyacrylamide running gels were used in this study.

Separation of glutenins by 2-DE

For sample preparation, three fully developed kernels were selected from each cultivar under each condition. The embryos were cut off and the endosperm was hammer-milled to a fine powder. The gliadins were removed by washing with aqueous ethanol and each sample solution (including glutenins and remaining gliadins) was then loaded on isoelectric focusing gel for the first dimension and equilibration according to O'Farrell (1975). For the second dimension, SDS-PAGE according to Tanaka *et al.* (2003) was performed with 15% polyacrylamide running gel.

Quantitative analysis of SSPs by electrophoresis

Protein bands in the gels of A-PAGE and SDS-PAGE, and protein spots in 2-DE gels were stained with colloidal Coomassie Brilliant Blue G-250 solution according to Dyballa and Metzger (2009).

The stained gels were scanned using a flatbed scanner and the protein band patterns and protein spot patterns were visualized. To measure the protein quantity (PQ), the band density and the spot density were normalized by KW and PC, and then analyzed using JustTLC software Version 4.5 (Sweday) and PDQuest software Version 8.0.1 (Bio-Rad Laboratories, Inc.), respectively. The density of each band/spot reflects the PQ, and is displayed by "intensity volume", where the unit is "count" in the software. The PQ represents the expression level of each SSP. Therefore, the sum of PQ in SDS-PAGE and A-PAGE was viewed as the total expression levels of SSPs. The total PQ of the LMW-GS fraction was further assigned to three fractions (LMW-GS B, C, and D-types) based on the ratios of the three fractions in 2-DE, due to the overlapping of bands in the LMW-GS fraction in SDS-PAGE. Each intensity ratio of HMW-GSs, LMW-GS B, C, and D, and gliadins was given by each PQ/the sum of PQ. The data were statistically analyzed using JMP software ver. 12.0.1 (SAS Institute).

Results

Relationship between kernel characterization and flour quality under heat stress conditions

We characterized the kernel shape using the metrics of kernel length (LEN), kernel width (WID), horizontal axis proportion (HAP), sphericity (SPH), and vertical perimeter (VP) (**Fig. 1A**). The kernel size was characterized by the projection area (AREA), section area (SEC), surface (SUR), volume (VOL), and kernel weight (KW) (**Fig. 1B**). Generally, heat stress reduced the kernel shape and size in all tested cultivars. All cultivars had significantly lower ($p < 0.01$) WID, HAP, AREA, SEC, VOL, and KW under heat stress conditions than under normal conditions. A significant reduction in WID and a slight reduction in LEN appeared as the significant reduction in HAP (=WID/LEN × 100). These results caused significant reductions in AREA and SEC, which ultimately lead to significant reductions in VOL and KW. Furthermore, shrinkage and wrinkling of seeds occurred, significantly reducing VP and SUR.

PC in the wheat flour significantly increased ($p < 0.01$) in all cultivars under heat stress (**Fig. 2A**), while SSV showed a different tendency (**Fig. 2B**). SSV in 'Imam' was significantly decreased, but was still the highest among all cultivars regardless of conditions. SSV in 'Norin 61' was also slightly decreased under heat stress conditions. SSVs in 'Condor', 'Tagana', and 'VYT11' were slightly increased under the heat stress conditions or not significantly different from the normal conditions. Notably, SSV in 'Bohaine' was significantly increased under heat stress conditions,

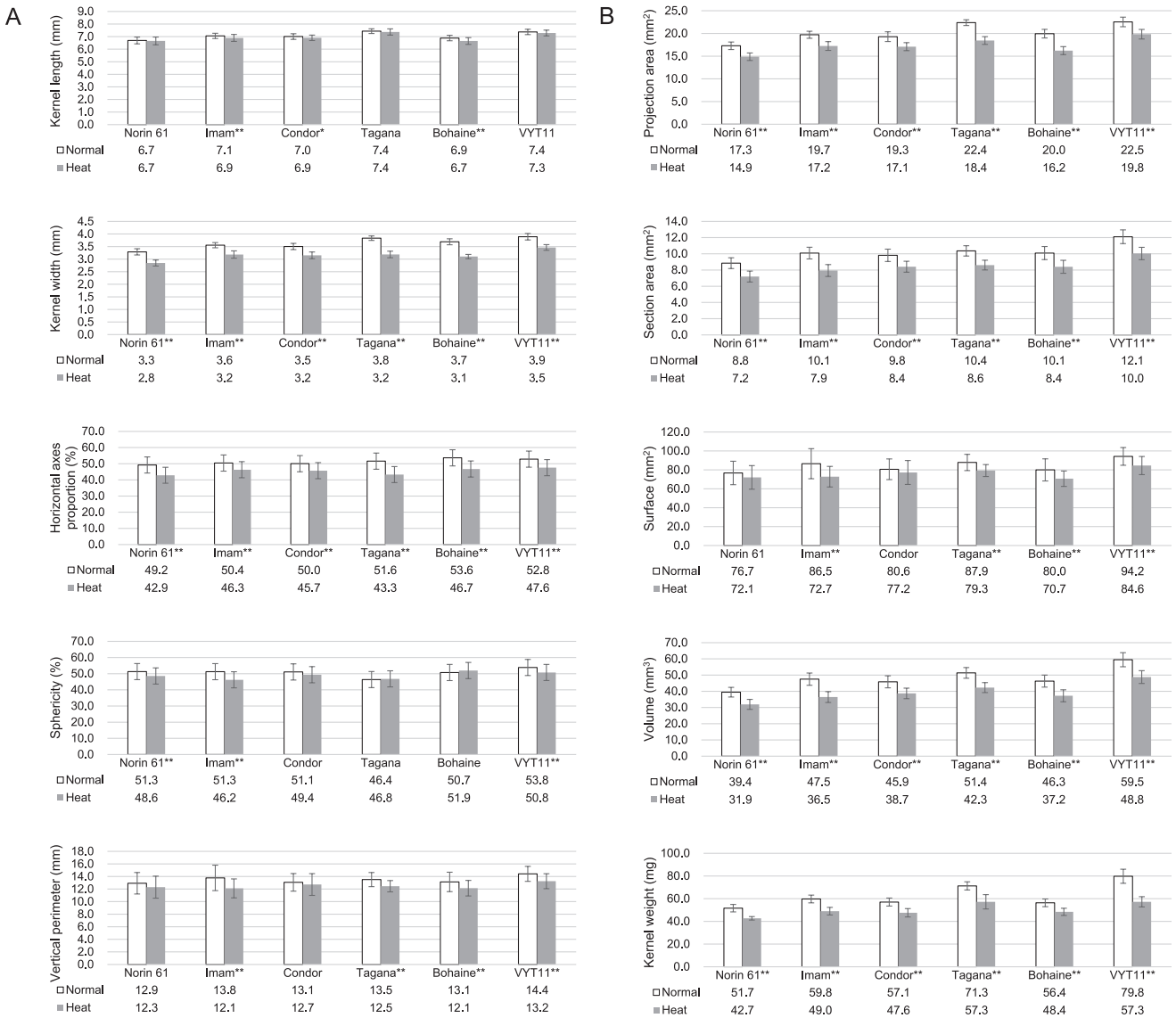


Fig. 1. Kernel characterizations of wheat plants under heat stress conditions. A: kernel shape traits. B: kernel size traits. Whiskers show standard deviations. Asterisks indicate a significant difference (* $p < 0.05$; ** $p < 0.01$).

indicating that the gluten strength under the heat stress conditions increased over that of the wheat under normal conditions.

Intensity ratios of SSPs under heat stress conditions

We fractionated the gliadins from the SSPs using alcohol, then further fractionated them into α-, β/γ-, and ω-gliadins by their mobility in A-PAGE (**Fig. 3A**). The other SSPs, which primarily consisted of glutenins, were fractionated into HMW-GSs and LMW-GSs by SDS-PAGE (**Fig. 3B**). In 'Imam', we found HMW-GSs '5 + 10', which are controlled by the *Glu-D1d* allele responsible for strong gluten, indicating suitability for bread making. To investigate the relationship of the intensity ratio of glutenins and corresponding dough strength, SSPs (primarily including glutenin) were further fractionated into four types: HMW-GSs, LMW-GS B-types (mostly typical LMW-GSs), LMW-GS C-types (mostly modified α- and β-gliadins), and LMW-GS D-types (modified ω-gliadins) by 2-DE (**Fig. 4**). In all cultivars under heat stress, the intensity ratio of total glutenin decreased, while the intensity ratio of total gliadins increased (**Fig. 5A**). The ratio of total LMW-GSs decreased, while the ratio of LMW-GS D-types, α- and ω-gliadins increased in all cultivars. Meanwhile, the ratio of HMW-GSs increased, and those of β/γ-gliadins decreased in 'Imam', 'Condor', and 'Tagana'. In addition, the ratios of LMW-GS B- and C-types also increased in 'Condor' and 'VYT11', respectively. As for the range of variation, 'Norin 61' had the largest variation (±3.59), and 'Condor' had the smallest variation (±0.43).

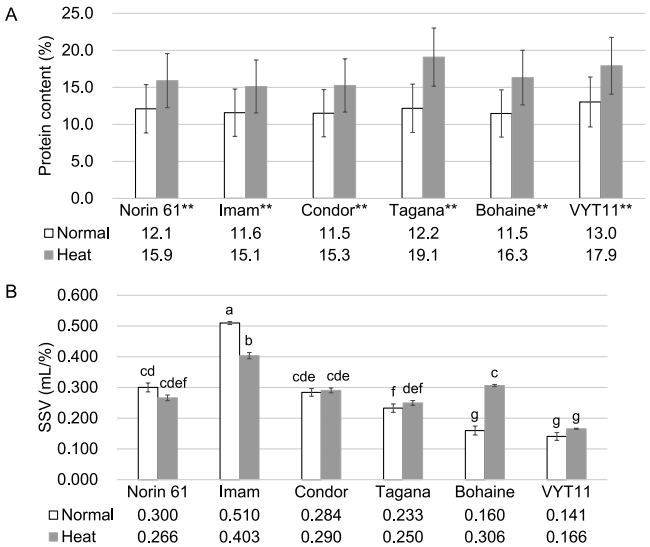


Fig. 2. PCs and SSVs of wheat plants under heat stress conditions. A: protein contents. B: specific sedimentation values. Whiskers show standard deviations. Same letters indicate no significant difference.

Sum intensity volumes of SSPs under heat stress conditions

Under heat stress, all cultivars displayed decreases in KW and increases in PC (**Figs. 1B**, **2A**). In 'Norin 61', 'Imam', 'Condor', 'Tagana', and 'Bohaine', the protein weight in single kernels (KW × PC) also increased, but the expression level reflected by intensity volumes of total SSPs in 1 mg of protein weight decreased (**Fig. 5B**). In 'Bohaine', KW × PC and the expression level of total SSPs increased. In 'VYT11', KW × PC decreased because of a significant decrease in KW and a decrease in the expression level of total SSPs.

According to the summed expression levels of glutenin and gliadin contained in SSPs, the levels of both fractions increased in 'Bohaine', but decreased in 'Norin 61', 'Imam', 'Condor', and 'Tagana'. The level of glutenin decreased, and the level of gliadin increased in 'VYT11'. The level of HMW-GS decreased in all cultivars except 'Bohaine'. The levels of all types of LMW-GS also increased in 'Bohaine'. These tendencies in 'Bohaine' were visually expressed in the intensity of the spots, differences in which were clearly evident between the normal and heat stress conditions (**Fig. 4**). Meanwhile, the level of LMW-GS D-types increased in all cultivars except 'Imam'. In addition, the levels of LMW-GS C-types and LMW-GS D-types increased in 'VYT11'. The levels of all fractions of gliadin increased in 'Bohaine' and 'VYT11', but decreased in 'Imam' and 'Condor'. The levels of α- and ω-gliadins increased in 'Tagana' and 'Norin 61', respectively, despite the decrease in total gliadin; this phenomenon was explained by the decrease in other gliadin fractions in both cultivars.

Discussion

In this study, we demonstrated that heat stress affects kernel traits, flour quality, and protein expression in some Sudanese common wheat cultivars, despite the "heat tolerant" classifications of these cultivars and their wide commercial adoption. The data indicated that the decreased VOL and KW of the wheat under heat stress were a result of narrow-shaped seeds. Starch (amylose and amylopectin) is the primary energy storage compound in wheat grains, comprising 60%–70% of dry weight in grains (Morell *et al.*

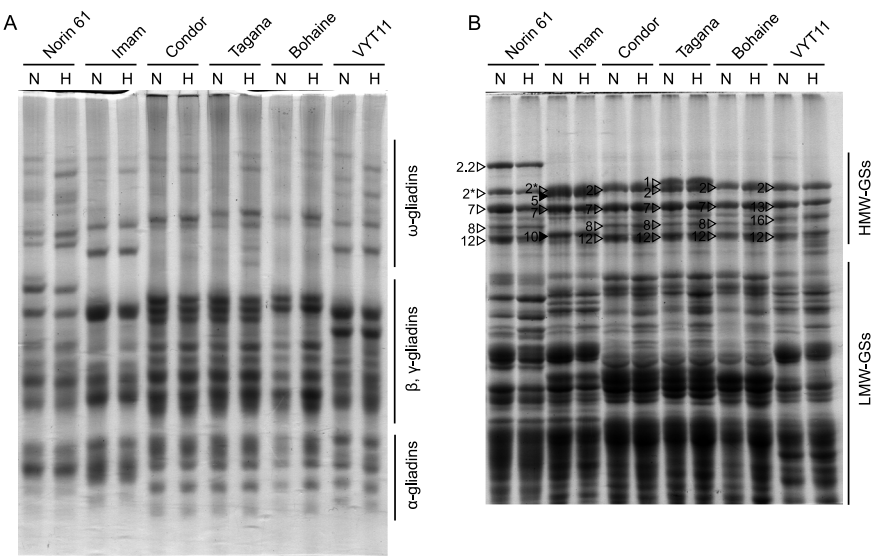


Fig. 3. Comparison of profiles of SSPs. Gliadin and glutenin profiles by A-PAGE (A) and SDS-PAGE (B). The positions of α-, β/γ-, and ω-gliadins are indicated to the right side of the gel in (A). The positions of the HMW-GS and LMW-GS are indicated to the right side of the gel in (B). The open arrowheads indicate HMW GSs. The subunit numbers of HMW-GSs are also indicated adjacent to the open arrowheads. The closed arrowheads indicate HMW-GSs '5 + 10'. N: normal conditions. H: Heat stress conditions.

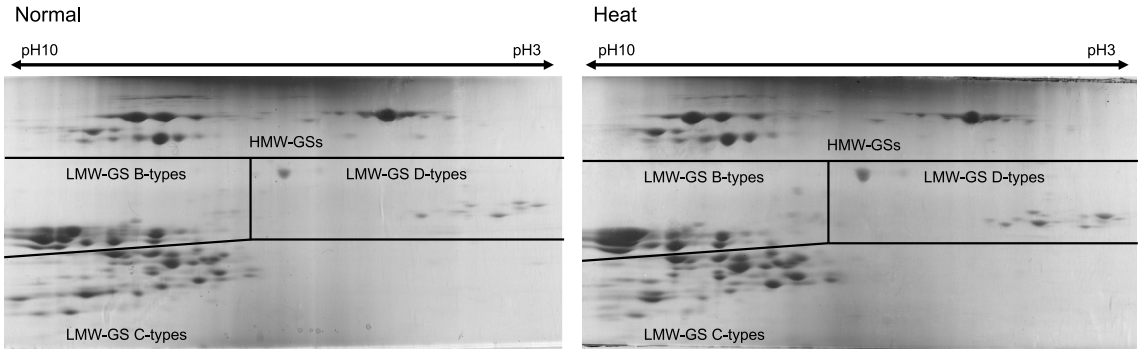
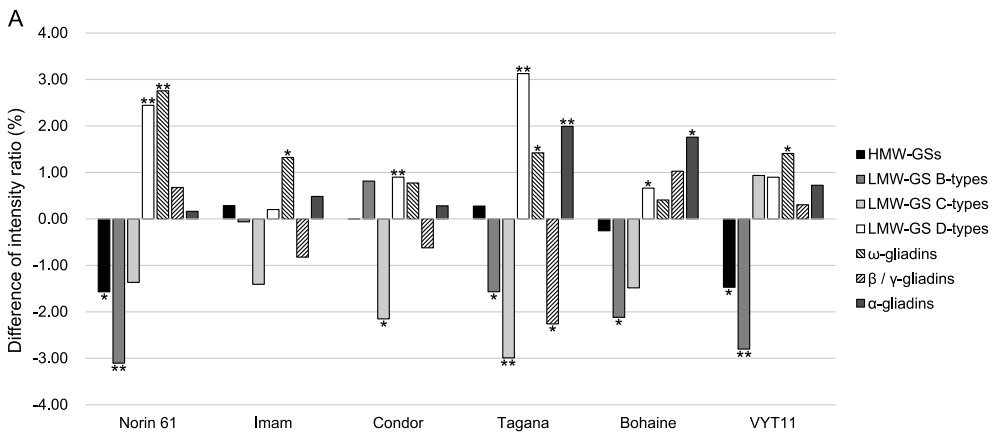
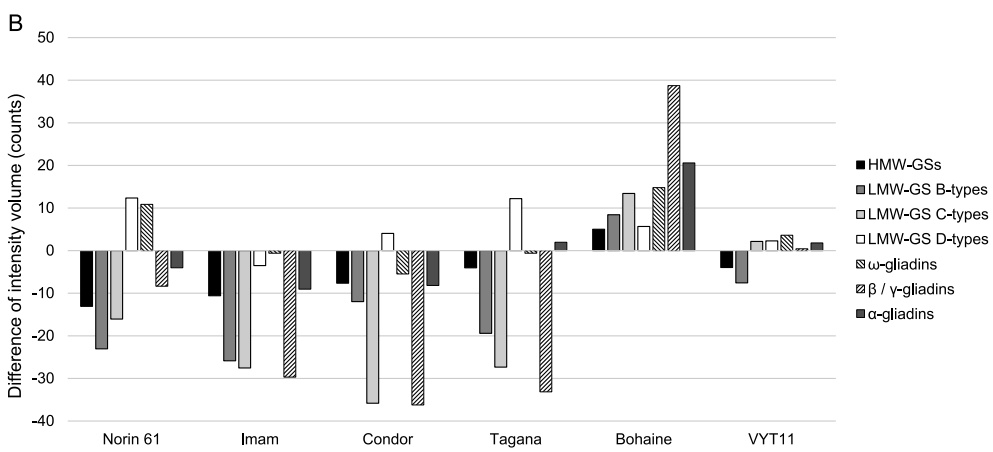


Fig. 4. Comparison of glutenin profile between normal and heat stress conditions in 'Bohaine' by 2-DE. Left and right gels are glutenin profiles in 'Bohaine' under the normal and heat stress conditions, respectively. Areas of HMW-GSs and LMW-GS B-, C-, and D-types are indicated in the gel.



	Norin 61	Imam	Condor	Tagana	Bohaine	VYT11
Total glutenins	-3.59	-0.98	-0.43	-1.15	-3.20	-2.44
Total gliadins	3.59	0.98	0.43	1.15	3.20	2.44



	Norin 61	Imam	Condor	Tagana	Bohaine	VYT11
Difference of KW × PC	0.54	0.49	0.70	2.25	1.43	-0.13
Total glutenins	-39.83	-67.49	-51.38	-38.57	32.54	-7.09
Total gliadins	-1.50	-39.33	-49.87	-31.81	74.11	5.83
Total SSPs	-41.33	-106.82	-101.24	-70.37	106.65	-1.26

Fig. 5. Comparison of expressions of SSPs. A: Different intensity ratios of SSPs. The difference of intensity ratios indicates the difference in heat stress conditions relative to normal conditions. The total glutenins or gliadins are the sum of the differences in intensity ratios. Asterisks indicate a significant difference (* $p < 0.05$; ** $p < 0.01$). B: Sum intensity volumes of SSPs. The difference of intensity volumes indicates the difference in heat stress conditions relative to normal conditions. The total glutenins, gliadins, and SSPs are the sum of the differences in intensity volumes.

1995). Yan *et al.* (2008) showed that under heat stress, total starch and amylopectin concentrations decreased significantly, but amylose concentrations increased in comparison with a control. Narrow-shaped seeds may therefore be due to amylopectin concentrations decreased from the 70%–80% of total starch in wheat grains grown under normal conditions. The starch composition of wheat is also an important determinant of flour quality; decreases in the amylopectin to amylose ratio might also affect the gelatinizing properties of the flour.

We investigated PC and flour quality. Heat stress significantly increased the PC in wheat flour made from all cultivars. These results may reflect a decrease in total starch in the wheat grain, despite the relatively constant or slightly decreased protein expression under heat stress conditions. Increases in PC are generally considered to make a strong dough and to increase bread loaf volume. However, our results showed that SSV, which is highly correlated with the dough strength, varied among the wheat cultivars. 'Imam' had the greatest decrease in SSV due to heat stress, although its value was still the highest among the wheat cultivars; this result could be due to the presence of the *Glu-D1d* allele in 'Imam'. Notably, several studies have shown that wheat varieties carrying the *Glu-D1d* allele are generally more tolerant to heat stress-induced declines in dough quality than those carrying the *Glu-D1a* allele (Blumenthal *et al.* 1995, Don *et al.* 2005, Irmak *et al.* 2008, Uthayakumaran *et al.* 2012). This suggests useful applications of the *Glu-D1d* allele in future improvement of end-use qualities in wheat grown under heat stress.

As for the intensity ratios of SSPs, we found that the gliadins to glutenins ratio increased in all cultivars under heat stress conditions. For heat stress, total protein per grain is the most functional criterion because it reflects only the synthesis of proteins, while the protein content (%) of wheat flour is more meaningful for wheat processing quality. Daniel and Triboi (2000) reported that an increase in average daily temperatures induced a decrease in total gliadins per grain, while the proportion (%) of gliadin in the total protein content remained stable (approximately 29%). This indicates that the increase in temperature induces the synthesis of gliadins and reduces the synthesis of glutenins. Consequently, the gliadins to glutenins ratio increases too much and the hydrated wheat flour dough becomes too more viscous, a property unsuitable for bread making.

We also found that the ratio of total LMW-GSs decreased under heat stress conditions in all cultivars. LMW-GSs are a major component of SSPs and account for 40% of SSPs in wheat flour grown under normal conditions. Graßberger *et al.* (2003) reported that the addition of reoxidized HMW-GSs caused an increase in dough extensibility and resistance; additional reoxidized LMW-GSs increased only the dough resistance. This indicates that the decreased content of the short-spring (LMW-GS) makes the dough more viscous than the normal content of LMW-GSs. In addition, the increased ratio of LMW-GS D-types, which are modified ω-gliadins, α- and ω-gliadins, also contributed to the viscosity of the dough. Meanwhile, opposite changes in the ratio were found in 'Imam', 'Condor', and 'Tagana', contribute to more elastic dough. For heat tolerance, the small variation in the ratio of each SSP seen in 'Condor' was considered to be a preferable character.

KW and PC are considered to be good indicators of heat stress response. Wardlaw *et al.* (1980) reported that the negative effect of temperature increases on the grain weight can be explained by the different behaviors of the carbon (C) and nitrogen (N) metabolisms. During grain filling, high temperatures increase the daily flow of C and N through the grain but decrease the flow of C per degree-day. In addition, Wardlaw *et al.* (1980) reported that the duration expressed in thermal time (degree-day) was relatively constant, while the duration as expressed in number of days was strongly affected. The quantity of C in the grain is therefore considered to be more affected by the temperature than the quantity of N. These reports could explain the decrease in KW and the increase in PC in the tested cultivars under heat stress conditions. In this study, KW × PC (indicating the protein weight in a single kernel) increased, even though the expression level of total SSPs decreased in all cultivars except 'Bohaine'. These results could be due to the increased expression of heat shock proteins (HSPs) to increase tolerance of heat stress, which may have supplanted the expression of SSPs. Notably, the expression level of total SSPs increased in 'Bohaine' even under heat stress conditions, indicating that 'Bohaine' may be insensitive to or tolerant of to heat stress; the expression level of HMW-GSs increased only in 'Bohaine'. Although HMW-GSs account for only about 10% of the total SSPs in mature seeds, multiple correlation coefficients have indicated that almost 80% of the variation in the Alveograph *w* value (a combined measure of dough strength and extensibility) can be accounted for by variations in flour HMW-GS composition and protein content (Payne *et al.* 1988). The HMW-GSs are therefore important for the bread-making process, and the increase in the expression level of HMW-GSs might contribute to dough elasticity, for a metric of bread-making quality. In fact, we observed an increase in SSV in 'Bohaine' even under heat stress conditions.

Among the Sudanese cultivars, 'Imam' currently possesses relatively desirable flour for bread making under heat stress conditions (Tahir *et al.* 2006). We found that this result is likely due to the *Glu-D1d* allele in 'Imam'. For further improvement of bread-making quality, we propose that the *Glu-D1d* allele be introduced to 'Bohaine', which has a genotype to maintain high SSP expression levels even under heat stress conditions. Furthermore, the addition of the 'Condor' character, which is a small variation in the ratio of each SSP, may provide an additional advantage.

Author Contribution Statement

HTanaka and HTsujimoto constructed the study concept. HTanaka designed the study and wrote the initial draft of the manuscript. HTanaka, YSAG, MF, HS and ISAT contributed to analysis and assisted in manuscript preparation. HTanaka, YSAG, ISAT and HTsujimoto have contributed to data interpretation, and reviewed the manuscript. All authors approved the final version of the manuscript.

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