Doctoral Thesis

Nutritional and physiological studies on improvement of productivity and grain quality in wheat (*Triticum aestivum* L.) under drought stress condition

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Abbreviations

AACC	American association of cereal chemistry
Ca	Calcium
DW	Dry weight
$F_{1, 2, 3….}$	Fertilizer levels
Hi	Harvest index
K	Potassium
Mg	Magnesium
N	Nitrogen
NDMC	National drought mitigation center
P	Phosphorus
Phy-P	Phytate phosphorus
Pi	Inorganic phosphorus
SA	Salicylic acid
SPSS	Statistical package for the social sciences
SSP	Single super phosphate
$T_{1,2,3}$	NPK levels
TCA	Trichloroacetic acid
TDR	Time domain reflectometer
TP	Total pentosan
WSP	Water-soluble pentosan
WUE	Water use efficiency
Zn	Zinc

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Chapter 1

General Introduction

1.1 Impact of drought stress on plant growth and crop yield

Stress is defined as an external factor that exerts detrimental influences on the plant and is measured in relation to plant survival, growth, crop yield, or the primary assimilation processes which are related to overall growth (Taiz and Zeiger 2006). Stress characteristics such as severity, duration, number of exposures, and combination of stresses; as well as plant characteristics like organ or tissue in question, stages of development and genotype determine survival and growth, or death of a given plant (Larkindale *et al*. 2005; Farooq *et al*. 2009). National Drought Mitigation Center (NDMC) has classified drought into five categories, namely, meteorological drought, agricultural drought, hydrological drought, socio-economic drought, and ecological drought. For the farming purpose, drought is understood as an agricultural drought which is supposed to have occurred when soil water is not available in enough amounts for normal growth and development of a crop at a particular time (NDMC, 2006). Plant wilting is the first detectable symptom of drought stress which indicates rapid transpirational water loss which exceeds the rate of water absorption (Buchanan *et al*. 2002). Drought stress and high temperature at flowering stage decreases grain-set due to lower fertilization which is caused by pollen sterility and ovule abortion (Hossain *et al*. 2012). Sangtarash (2010) found greater decline in the grain number when drought occurred at or immediately after anthesis. Seed abortion in winter wheat varieties was observed when drought stress was applied at 5-9 days after anthesis; a higher seed abortion incidence was noted in a drought sensitive cultivar compared to a tolerant cultivar (Fabian *et al*. 2011). Drought stress at post anthesis stage declines grain yield by decreasing individual grain weight (Ahmadi and Baker 2001; Ji *et al*. 2010). The reason for this lower individual grain weight may be due to short duration of grain filling (Wardlaw and Willenbrink 2000; Prasad *et al*. 2008). Hexaploid and diploid wheat varieties subjected to drought stress for 10 days at 50, 60 and 70 days after sowing, markedly declined its chlorophyll and carotenoid content (Chandrasekar *et al*. 2000). Drought stress enhances chlorophyll degradation, lipid peroxidation, and nitrogen loss which results in leaf senescence (Yang *et al*. 2001), and increased leaf electrolyte leakage (Liu *et al*. 2006). Drought stress at anthesis stage causes pollen sterility, and thus reduces seed-set. Wheat cultivars grown under rainfed condition usually face drought stress, and significant yield loss and that periodic drought often affected 50% and 70% of wheat areas in developing and developed countries, respectively (Trethowan and Pfeiffer 1999).

1.2 Effect of drought stress on grain protein and starch

Protein is an important component of wheat grain that determines bread making quality (Pomeranz 1987; Shewry 2009). Crude protein content in the matured wheat grain varies from 10-20% (Shewry *et al*. 1995). Loaf volume of bread is affected by protein quantity, in hard red winter and spring wheat cultivars (Finney and Barmore 1948). Quantity and quality of protein affect bread making properties like water absorption capacity, oxidation requirements, loaf volume and crumb characteristics (Finney 1984). A high protein content increases water absorption in the flour resulting in a larger loaf volume and softer bread. Bread with a higher protein content can be stored longer (Maleki *et al*. 1980). Grain yield potential reduces in response to drought stress at grain filling period, and thus the grain size is decreased showing higher content of protein (Fowler 2003). Nitrogen (N) fertilization enhances crude protein content of wheat, and application of N fertilizer later in the season (post-anthesis stage) was more effective compared to earlier application in protein quality improvement (Wang *et al*. 2008). Application of N fertilizer at later growing season compensates the negative effects of excessive precipitation on protein accumulation (Zhang *et al*. 2009; Zhao *et al*. 2009). Starch is the major constituent of wheat grains, ranging from 54 to 72% of grain dry weight, which affects the structure of baked products (Hoseney *et al*. 1978). Drought at post-anthesis period can reduce the number of endosperm cells and starch granules per cell (Nicolas *et al*. 1985). Drought stress during grain filling stage often inhibits the conversion of sucrose into starch, and therefore prevents starch accumulation in the grain (Xie *et al*. 2003).

1.3 Role of N, P and K in alleviating drought stress

Nitrogen (N) is one of the main macro nutrients, that enhances plant growth, increases leaf size and quality, improves seed set and development and it is a key component in proteins and enzymes (Brady and Weil 2008; Foth and Ellis 2010). Increased doses of N fertilizer can improve drought tolerance in plants (Halvorson and Reule 1994). Application of N improves water use efficiency and mitigates the negative effects of drought stress on plant growth (Saneoka *et al*. 2004). Zhou and Oosterhuis (2012) concluded that N fertilization can enhance drought tolerance by promoting the activity of antioxidative enzymes, which helps in decreased lipid peroxidation and improved root vigor. N fertilization under drought stress condition can improve chlorophyll content and photosynthetic activity by increasing leaf area, and therefore promoting photosynthetic efficiency and mitigating photo damage under water stress (Wu *et al*. 2008). Under drought stress condition, malondialdehyde concentration increases in the leaves however, supplemental application of N decreases malondialdehyde concentration in both drought-stressed and well-watered plants (Saneoka *et al.* 2004).

Phosphorus (P) plays a key role in preserving and transferring of energy in plant metabolism (Wang *et al.* 2015), and is a constituent of phospholipids, nucleic acids, and adenosine triphosphate (Amtmann and Blatt 2009). Application of P positively affects plant growth under drought stress by enhancing root growth (Singh and Sale 1998), water use efficiency (Garg *et al.* 2004; Waraich *et al*. 2011), leaf area and photosynthetic activity (Singh *et al.* 2006), leaf stomatal conductance and nitrate reductase activity (Bruck *et al*. 2000; Naeem and Khan 2009**)**, cell membrane stability and water relations (Sawwan *et al*. 2000; Kang *et al.* 2014). Singh and Sale (1998) observed that leaf phosphorus content was significantly increased in plants growing under water stress condition compared to well-irrigated. Water content of fresh leaves and individual leaf area are significantly influenced by P content of leaves (Singh *et al*. 2006). Applying P fertilizer deep into soil is a feasible and practical means for obtaining a higher grain yield and improved water use efficiency (WUE) in winter wheat grown in semiarid regions (Kang *et al.* 2014).

Potassium (K) is an influential macro-nutrient which plays a key role in improving plant water status, stomatal movements, enzyme activity, osmoregulation and membrane stability (Ahmad *et al*. 2014; Jatav *et al*. 2014; Erel *et al*. 2015)**.** An adequate application of K fertilizer may improve WUE, and thus plant growth under drought stress condition (Eakes *et al.* 1991; Egilla *et al*. 2001; Jatav *et al*. 2014). K application can increase leaf water potential, turgor potential and cell membrane stability, and decrease stomatal resistance. An adequate level of K improves plant productivity by maintaining osmotic balance and influencing leaf characteristics such as leaf water content and thickness (Premachandra *et al*. 1991). K fertilization maintains the activity of aquaporins and improves osmotic adjustment, thus it enhances water uptake, promotes root growth, cell membrane stability, cell elongation, stomatal regulation and detoxification of reactive oxygen species (ROS) resulting in improved drought stress tolerance (Wang *et al.* 2013). Increased levels of K nutrition decrease ROS and thus helps to protect cells from ROS induced oxidative injury, resulting in maintained photosynthetic electron transport (Cakmak 2005). Under drought stress condition, the stomata function is negatively affected in K deficient plants, and resulted in greater water loss. Therefore, an adequate level of K nutrition can improve plant drought tolerance by enhancing water relations and WUE resulting in a better plant growth (Wang *et al*. 2013).

1.4 Role of salicylic acid (SA) in mitigating the effects of drought stress

Salicylic acid (SA) is a phenolic compound that plays a key role in photosynthetic activity, stomatal conductance and transpiration (Arfan *et al*. 2007), and enhancing anti-oxidative protection (Xu *et al*. 2008). Foliar application of SA regulates the activities of antioxidant enzymes and increases plant tolerance to abiotic stresses (Erasalan *et al*. 2007). SA application can modulate important enzymatic and non-enzymatic components of AsA–GSH (ascorbate-glutathione) pathway, and also glyoxalase system (Gly I and Gly II) and decrease oxidative stress in drought-exposed plants (Alam *et al*. 2013). SA importantly contributes to growth and development regulation, although the biochemical mechanisms that mediate most of these responses remain largely unknown (Mariana *et al*. 2011). It has been proposed that SA affects the plant growth under stress through affects in nutrient uptake, water relations, stomatal regulation and photosynthesis (Hayat *et al*. 2009). Pirasteh *et al*. (2012) concluded that exogenous application of SA may enhance plant performance under drought stress by modulating various physiological and biochemical processes which are negatively affected by drought stress.

1.5 Study materials

Wheat (*Triticum aestivum* L.) is one of the major staple food crops that makes a significant contribution to global food production and food safety particularly in developing countries (Xia *et al*. 2012). Wheat can adapt to different climatic conditions, it has multiple end-uses, and dynamic nature of genomes and polyploidy character, that made it a nutritionally and financially important crop (Dubcovsky and Dvorak 2007). Wheat flour is used to prepare bread, confectionary products, biscuits, noodles and vital wheat gluten. It is also used for ethanol production, brewing of wheat beer and wheat protein in meat substitutes. Wheat bran and wheat germ could be reliable sources of dietary fiber that can prevent some digestive disorders (Campillo *et al.* 2010). Wheat whole grain contains antioxidants that help with reduced risk of some types of cancer. Wheat antioxidants suppress intestinal tumor activity in Min mice (John *et al*. 2006). Morris *et al*. (1991) revealed that approximately 32 % of wheat cultures suffer from several types of drought stress during the growth season in developing countries. In Afghanistan rain fed wheat faces a common problem of water shortages during grain filling period which results in low productivity and yield loss. An attempt was made to overcome this problem by introducing drought tolerant wheat varieties, however yield loss due to drought stress is still unavoidable.

1.6 Study objectives

Keeping in view the detrimental effects of drought stress on plants and the influential role of NPK fertilization and exogenous application of SA in mitigating these effects, the present study was conducted with the following objectives:

1- To assess the effect of NPK fertilization on agronomic performance, productivity, grain mineral content, starch, crude protein, total and water soluble pentosan and phytate P content of wheat under normal (non-stressed) condition.
2- To evaluate the effect of mother plant NPK nutrition on seed germination, and physiological performance of wheat during germination period.
3- To determine the effect of NPK fertilization on wheat growth, grain yield, and grain nutritional quality under drought stress condition.
4- To find out the effect of combined application of SA and different levels of K fertilization on productivity and nutritional quality of wheat under drought stress condition.
5- To determine how to improve drought tolerance and enhance wheat productivity and grain nutritional quality under drought stress condition.

Chapter 2

Effect of NPK fertilization on grain yield, nutritional and anti-nutritional quality, and seed germination of wheat

2.1 Introduction

Sustainable agricultural productivity and ensuring food security is possible through a precise and wise management of nutrients. Adequate application of fertilizers enhances the yield per unit area and improves grain quality and bread quality of wheat. N is an important component of proteins, nucleic acids, enzymes, coenzymes, and chlorophyll, and it contributes to the biochemical processes of the plant (Benin *et al*. 2012). N fertilization at anthesis, is more effective in the synthesis of a high grain protein content than an earlier application (Wuest and Cassman 1992). Sufficient N fertilization results in the production of higher productive tillers and increased number of spikes, number of grains per spike, grain yield, and biological yield. P fertilization of wheat crop has significantly increased the plant height, number of tillers per plant, the straw and grain yield, and P uptake by grains (Alam *et al*. 2003). More than 70 % of the total P is stored in the grain as phytate (Rosa 1999). Phytate (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate) is an anti-nutritional factor that binds with proteins and some important micronutrients, such as iron and zinc, and significantly reduces the availability of these nutrients (Raboy 2001). Accordingly, it is necessary to reduce the content of such anti-nutrient compounds in wheat grains by proper nutrient management. K plays a vital role in the biochemical functions of the plants like improvement of protein and carbohydrates, activating various enzymes, enhancement of fat content, developing drought tolerance, and resistance to lodging and frost (Marschner 1995). An optimum dose of K increases the number of effective tillers, grains per spike, 1000 grain weight, grain yield, straw yield, and protein content of wheat (Alam *et al*. 2009). Pentosan is a major fiber component of the non-starch polysaccharides in cereal, which is called flour gum or hemicellulose. It affects food absorption in the human body, and decreases absorption of lipids and cholesterol and, therefore, plays a key role in the human diet (Mohammadkhani 2005). Pentosan is an important component of dough in which they bind water and contribute to the formation of viscous dough (Buksa *et al*. 2010). Water soluble pentosan has a positive effect on the bread-making quality of wheat flour (Courtin and Delcour 2002). Gluten is composed of glutenins and gliadins, which play important roles in the baking quality of bread due to their influence on the water absorption capacity, elasticity, and extensibility of dough and, thus, affect the flour quality of wheat (Torbica *et al*. 2007). Phytase (*myo*-inositol hexaphosphate phosphohydrolase) is an enzyme which catalyzes the hydrolysis of Phy-P to inositol and orthophosphate and helps the bioavailability of phosphorus. Seed germination increases phytase activity by *de novo* synthesis of this enzyme during germination (Sung *et al*. 2005).

The objectives of this chapter were to evaluate the effects of NPK fertilization on growth, grain yield, grain quality, and anti-nutrient content, and the influence of mother plant NPK nutrition on the seed germination, establishment, and physiological changes during seed germination in wheat.

2.2 Materials and methods

2.2.1 Plant materials and growth conditions

The wheat cultivar Minaminokaori was grown in a vinyl greenhouse to prevent nutrient leaching due to rainfall with natural sunlight and temperature in Hiroshima University. Regosol and nursery soil contained compost (Forex Torin Co. LTD. Japan) were mixed in a ratio of 2:1, and filled in containers (1.5 m in length, 30 cm in width, and 18 cm in depth). Chemical analysis of mixture soil showed that it contained: 0.20 % total N, 6.84 mg kg^{-1}

available P, and 79.85 mg kg^{-1} available K. Furthermore, dolomitic calcium magnesium carbonate was mixed (at a rate of 1 ton ha^{-1}) with the soil to adjust the pH (H_2O) to 6.5.

This study comprised with a control, where no fertilizer was applied (T_1), and two levels of NPK fertilizer: T_2 (110 kg N + 60 kg P_2O_5 + 55 kg K_2O ha^{-1}), and T_3 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}). The source of NPK was urea, single super phosphate, and potassium chloride, respectively. All of P and K, and a half dose of N were applied before sowing, and the remaining N was applied in two equal splits at the tillering and anthesis stages. Wheat seeds were sown in the third week of November 2015 and 10-day-old seedlings were transplanted into the containers at a 10 cm-distance, following a randomized complete block design with 4 replications. Normal recommended agronomic practices were followed during the experimental process.

2.2.2 Plant harvesting, grain yield, and measurement of yield components

Plants were harvested, at the end of May 2016, when all spikes were completely matured. Number of tillers per plant was obtained by counting all tillers in 5 random plants from each treatment, before harvest. Number of spikes per plant was obtained by counting spikes in 5 random plants from every treatment. To measure 1000 kernel weight, 500 grains were counted, weighted with a prescribed accuracy and the value was multiplied by 2. To ascertain Grain yield, wheat plants were harvested after maturity, the spikes were oven dried at 80 ^{0}C for 48 hours, threshed, and grain yield was recorded and expressed as kg ha^{-1}.

2.2.3 Determination of grain mineral content

Samples of mature seeds were ground finely with a vibrating sample mill (TI-100, Heiko, Japan) for the measurement of grain mineral content. Finely ground samples were digested by H_2SO_4 and H_2O_2 and K content was measured using a flame photometer (ANA 135, Tokyo Photoelectric, Tokyo, Japan). Ca, Mg, and Zn were measured by an atomic absorption flame emission spectrophotometer (AA-6200, Shimadzu, Japan). The total P was determined by a UV-spectrophotometer (U-3310, Hitachi Co. Ltd. Tokyo, Japan) following the molybdenum reaction solution method suggested by Chen *et al*. (1956). Grain inorganic Phosphorus (Pi) was extracted in trichloroacetic acid (12.5%) + $MgCl_2$ (2 mmol /l) while stirring overnight, and Pi was measured colorimetrically (Raboy and Dickinson 1984). The total N was measured using the Kjeldahl method (Jones 1991) after sample digestion with concentrated H_2SO_4 and H_2O_2.

2.2.4 Determination of grain starch and crude protein content

For the determination of grain starch, 80% ethanol was added to the flour samples to remove sugars and then starch was extracted with perchloric acid. Anthrone reagent was added to the tubes containing extracted samples and then heated in a boiling water bath. The absorbance was measured at 630 nm (Nag 2016). Crude protein was calculated by multiplying the value of grain total N content by 5.47 (Fujihara *et al*. 2008).

2.2.5 Determination of grain TP, WSP and dry gluten content

Grain TP was measured following the orcinol-HCl method. Finely ground samples were hydrolyzed with 2 N HCl in boiling water for 2.5 hours, and centrifuged. Then a specific amount of the supernatants was transferred to new test tubes and reaction regents ($FeCl_3$ and Orcinol) were added and vortexed. The tubes were heated in boiling water for 30 minutes, cooled, and the absorbance was measured using a spectrophotometer. WSP was extracted by hydrolyzing flour samples in distilled water with shaking for 2 hours at 30°C. Then, 4 N HCl was added to the aliquots of the supernatant and placed in boiling water for 2 hours and allowed to cool. WSP was estimated by a spectrophotometer, using $FeCl_3$-orcinol reagents (Hashimoto *et al.* 1986). Gluten content was measured according to AACC international approved method (38-10.01), by hand washing with 30 minutes resting time, and the result was expressed as dry gluten percentage.

2.2.6 Determination of grain Phy-P content

Phy-P was measured following the method suggested by Raboy and Dickinson (1984) where aliquots of flour were extracted in extraction media (0.2 M HCl: 10% Na_2SO_4) overnight at 4°C while shaking. Extracts were centrifuged, and Phy-P was obtained as a ferric precipitate and assayed for P using ammonium molybdate reaction reagent.

2.2.7 Seed germination experiment

To determine the effect of mother plant NPK fertilization on the growth and physiological performance of produced seeds, 200 seeds with 4 replicates were planted on wetted germination papers (top of paper method) and placed in a germinator at 23°C for 7 days. Germinating seed samples were taken every day, frozen with liquid N, and stored under -80°C. The data on phytase activity, Phy-P and Pi contents were recorded daily.

2.2.7.1 Seed germination test and determination of physiological attributes

Normal seedlings were counted on the 7th day of germination and the result was expressed in percentage. Seedlings were harvested on the 7th day of germination and fresh weight was recorded. To measure phytase activity, fresh samples were ground with liquid nitrogen, transferred to Erlenmeyer flasks, and a buffer solution (Na-Phytate + Sodium acetate) was added. Then, the samples were shaken for 30 minutes at 37°C. Subsequently, aliquots of the sample were transferred to two sets of plastic tubes and placed in a water bath at 37°C. The Phytase activity was stopped by adding trichloroacetic acid (TCA) to the first set of test tubes to act as a control, then, TCA was added to the second set of test tubes after 30 minutes to stop enzyme activity. The test tubes were centrifuged, and the supernatants were transferred to new test tubes, and reagent solutions (ammonium molybdate + ferrous sulfate heptahydrate) were added. The absorbance was measured colorimetrically at 700 nm against the control (Eeckhout and Paepe 1994). Determination of Phy-P and Pi were carried out following the same procedure as mentioned previously, and the result was expressed based on the dry weight.

2.2.8 Statistical analysis:

All the collected data were subjected to analysis of variance using the SPSS statistics package, version 21 (IBM Inc., USA) and means ($n = 4$) were separated using the Duncan multiple range test at $p < 0.05$.

2.3 Results

2.3.1 Number of productive tillers per plant

Mean number of productive tillers per plant was ranged from 5.5 to 13.35. The highest number of tillers per plant was observed in T_3. However, T_1 (control) recorded the lowest number of productive tillers per plant (Table 2.1). NPK fertilization increased the number of productive tillers per plant by 145.0% in T_3 and 77.4% in T_2 compared to control, respectively.

2.3.2 Number of grains per spike

The maximum number of grains per spike was recorded in plants with a high rate of NPK fertilization (T_3). While, the lowest number of grains per spike was recorded in T_1. NPK fertilization increased number of spikes per plant compared to control by 11.3 % in T_3 and 6.6 % in T_2 respectively (Table 2.1).

2.3.3 1000 grain weight (g)

1000 grain weight which is an important yield component was significantly higher in plants which were supplied with a high rate of NPK. T_3 recorded the highest 1000 grain weight (Table 2.1) and the lowest grain weight was observed in T_1 (control).

2.3.4 Grain yield

Grain yield was significantly affected by the various levels of NPK fertilization. Application of a high rate of NPK (T_3) resulted in an increase in the number of spikes per plant and the kernel weight, which eventually contributed to a higher grain yield. It was observed that NPK fertilization, increased grain yield by 151.6 % in T_3 and 81.59% in T_2 compared to T_1 (Table 2.1).

2.3.5 Grain mineral content

Statistical analysis of the data showed that the contents of grain minerals (N, P, K, Pi, Mg, Zn, and Ca) were highly affected by NPK fertilization (Table 2.2). Grain minerals were found to be significantly higher in plants supplied with a high dose of NPK (T_3), while the lowest grain mineral content was observed in the control plants (T_1). A high rate of NPK fertilizers (T_3) increased the content of N by 65.2, P by 33.9, K by 8.9, Pi by 80, Mg by 19.7, Zn by

26.1, and Ca by 166.0% compared to the control, respectively. Application of a moderate rate of NPK (T_2) increased grain N, P, K, Pi, Mg, Zn, and Ca content by 31.8, 20.0, 3.6, 49.4, 8.3, 17.8, and 51.4% compared to the control, respectively.

2.3.6 Grain starch and crude protein content

The starch content was decreased with an increase in the NPK application rate however, the difference was not statistically significant. The highest crude protein content was observed in T_3 plants, followed by T_2 plants and the lowest crude protein was recorded in T_1 where no fertilizer was applied (Table 2.3). There was a linear increase in the crude protein content with an increase in the NPK level. Grain crude protein content was increased by 65.3% compared to the control in the highest level of NPK application (T_3), where as a moderate increase of 31.8% was observed with T_2 plants compared to the control.

2.3.7 Grain TP, WSP and dry gluten content

Total pentosan (TP) content was significantly higher in T_1 (control) plants where no fertilizer was applied. With an increased level of NPK, the content of TP was decreased compared to the control by 15.6 and 9.7% in T_3 and T_2 plants, respectively. In contrast, WSP content was highest in T_3 plants. It was observed that NPK fertilization increased the WSP content by 40.5 and 20.7% in T_3 and T_2 plants, respectively, compared to the control (Table 2.3). The gluten content was significantly influenced by NPK fertilization. There was a linear increase in grain gluten content with an increase in the NPK fertilizer level. The highest rate of NPK fertilization (T_3) increased the grain gluten content by 5-fold compared to the control and moderate rate (T_2) increased it by 2.3-fold (Table 2.3).

2.3.8 Grain Phy-P content

The Phy-P content was influenced by NPK fertilization (Table 2.3). The result obtained from this study indicated that T_3 had a slightly higher grain Phy-P content compared to the control (T_1).

2.3.9 Effect of mother plant NPK nutrition on seed germination and physiological performance of the second generation

The final count of normal seedlings on the 7th day of germination showed that seedlings which were produced by seeds from T_3 and T_2 plants recorded a higher germination percentage compared to T_1 (Fig. 2.1). The seedling fresh weight was higher in both T_3 and T_2 seedlings over the control (T_1) on the 7th day of germination. There was no significant difference between T_3 and T_2 plants in fresh weight (Figure 2.2).

The phytase activity significantly increased during germination of both T_3 and T_2 treated seedlings and control (T_1) seedlings. A lower phytase activity was recorded in 0-day seeds before germination. The level of phytase activity was highest on the 6th day of germination and the phytase level was recorded as being higher in T_3 and T_2 seedlings compared to T_1 (Fig. 2.3). Germination enhanced the phytase level by 3.22, 3.38 and 4.25-fold in T_3, T_2, and T_1, respectively, on the 6th day of germination compared to 0-day.

The Phy-P content of the seeds significantly declined during the germination period. The highest Phy-P content was recorded in the seeds of T_3, followed by T_2 and T_1 plants before germination (0-day). The lowest Phy-P content was observed in T_1, followed by T_2 and T_3 seedlings on the 7th day of germination. At the 7th day of germination, the Phy-P content was decreased by 2.31, 2.34 and 2.43-fold for T_3, T_2, and T_1 compared to 0-day, respectively (Fig. 2.4).

Seed germination enhanced the phytase activity and resulted in bioavailability of inorganic P (Pi). There was a liner increase in Pi with increased time of germination (Fig. 2.5). The highest Pi was recorded in T_3 (2.09 mg g^{-1} dry weight), followed by T_2 (2.03 mg g^{-1} dry weight), and T_1 (1.73 mg g^{-1} dry weight) on the 7th day of germination, while the lowest Pi was observed in T_1 before germination (0-day).

2.4 Discussion

The results from this study indicated that combined NPK fertilization increased number of productive tillers, number of grains per spike, 1000 grain weight, grain yield, mineral content in seeds, grain quality, seed germination and physiological performance of germinating seedlings. Application of a high rate of NPK (T_3) enhanced plant growth and productivity, and resulted in a higher grain yield. These results are in accordance with Hussain *et al*. (2002), Laghari *et al.* (2010), and Abdel-Aziz *et al*. (2016) who concluded that the grain yield of wheat and cereal crops increased with the application of N, P, and K fertilizers.

The grain mineral content was significantly influenced by NPK fertilization. It was observed that the mineral content was increased with an increase in NPK rate. Laghari *et al*. (2010) and Campillo *et al*. (2010) concluded that application of N, P, and K enhanced the content of these mineral in wheat. Saha *et al*. (2014) reported that the application of P fertilizer (single superphosphate) enhanced the P content in wheat grain.

Grain quality, except for starch, was highly influenced by NPK fertilization. The starch content was not affected significantly by NPK fertilization, and a slight decrease in the starch content was observed with an elevated rate of NPK. There is a negative relationship between crude protein and starch content. N fertilization decreases the starch content of wheat grain (Kindred *et al.* 2008). Crista *et al*. (2012) and Hlisnikovsky and Kunzova (2014) reported similar findings that grain starch was higher in control plants where no fertilizer was applied. NPK fertilization was found to enhance the synthesis of the raw protein in wheat (Crista, 2012). Sameen *et al*. (2002) found that the crude protein content of wheat grain was increased due to application of a higher level of NPK fertilizers in wheat variety Inqulab 91. The effect of NPK fertilization on the grain TP and WSP contents of wheat has not been reported sufficiently in earlier research studies. The influence of ecological environment was found to be significant on the pentosan content of wheat grain (Chunxi *et al*. 2002). Increased pentosan content was found with additional N fertilization under water logging condition in waxy wheat (Jing *et al*. 2010). In this study, TP content was decreased with high NPK fertilization, whereas WSP was significantly increased. Courtin and Delcour (2002) reported that WSP had a positive impact on the bread-making quality of wheat, and that impact of water-unextractable pentosan was a negative. NPK fertilization significantly influenced the gluten content of wheat flour and the highest gluten content was recorded in T_3 where high rate of NPK was applied. Tanacs *et al*. (2005) also found that application of NPK fertilizers significantly increased the gluten content of 4 tested wheat varieties in all 3 years of investigation. Gaj *et al*. (2013) also found that mineral fertilization increased the gluten content of wheat compared to a control, however different levels of P and K did not affect grain gluten content significantly. Phy-P is the major storage form of P in cereals and therefore the content of Phy-P mostly depends on grain total P. The Phy-P content of many crops was determined by researchers (Garcia-Estepa *et al*. 1999; Rosa *et al*. 1999). However, there was no sufficient review on the effect of NPK fertilization on the Phy-P content of wheat grain. Application of a high rate of P fertilizer might be one of the reasons for high phytate content (Raboy and Dickinson 1984). In another study, Ali *et al*. (2014) found that application of P fertilizer increased grain total P content in wheat. Similarly, Laghari *et al*. (2010) revealed that NPK fertilization resulted in a higher P uptake of wheat. Phytase helps Phy-P metabolism and Pi bioavailability and activity of phytase was increased with NPK fertilization.

Mother plant NPK nutrition improved seed germination, seedling growth, and seed physiological performance. Higher nutrient reserves in seeds produced by NPK fertilized plants may be the reason for better physiological activity and a higher germination percentage in T_3 and T_2 seeds compared to T_1. Seeds of plants which received a higher level of fertilizers and irrigation during the production stage could be capable of having an increased seedling establishment ability (Hampton 1992). Similarly, Bittman (1989) found that germination percentage of seeds could be positively related to the amount of stored nutrients in the endosperm. Doddagoudar *et al*. (2004) concluded that application of a higher rate of NPK improved seed quality and resulted in a higher seed germination percentage in China aster (*Callistephus chinensis* Nees. L.). The nutrients present in the endosperm are hydrolyzed during seed germination to guarantee seedling establishment (Shimizu and Mazzafera 2000). In this study, NPK fertilization level of the mother plant improved grain nutrient reserves which helped for a better growth of seedlings and it contributed to a high seedling fresh weight compared to the control. Phytase activity reached a maximum level on the 6th day of germination and therefore higher values of phytase content were recorded in T_3 and T_2. Ma and Shan (2002) reported that seed germination significantly increased phytase activity by 2.04-fold on the 3rd day of germination in wheat. Adequate studies on the effect of NPK fertilization on phytase activity in germinating seeds has not been found. The higher P and protein content in the grains of mother plants (T_3 and T_2) compared to the control (T_1), may have contributed to the higher phytase level in T_3 and T_2 during the germination period. Sung *et al*. (2005) revealed that the increase in phytase level may be due to *de novo* synthesis of the enzyme during germination. It was observed that phytase level started to decrease slightly on the 7th day of germination in this study. This could be attributed to the degradation of this enzyme by active protease (Houde *et al*. 1990). There was a negative relationship between phytase activity and Phy-P content; as phytase activity increased the Phy-P content was decreased in seeds. The effect of NPK fertilization on the seed phytate content during germination has not been studied before. Phytate is degraded by phytase during the seed germination of cereals (Kumar *et al*. 2010). The same trend in the reduction of content in germinating seeds of cereals has already been reported by other researchers (Azeke *et al*. 2011; Sokrab *et al*. 2012). The inorganic P (Pi) content was increased during the germination period and it was at maximum on the 7th day of germination. The influence of NPK fertilization on Pi content during seed germination has not been reported yet. In germinating seeds phytase removes orthophosphate groups from the inositol ring of phytate to produce free Pi, and a chain of intermediate myo-inositol phosphates (Debnath *et al*. 2005). The increase in the phytase activity of germinating seeds, which coincides with a decrease in the phytate content, may enhance P availability and utilization (Azeke *et al*. 2011).

2.5 Summary

In this chapter, the effect of NPK fertilization on growth of wheat, grain yield, grain mineral content, grain quality, contents of gluten, pentosan, and Phy-P in seeds and influence of mother plant NPK fertilization on the seed physiological attributes during germination period were studied. The results indicated that a higher level of NPK (T_3) fertilization enhanced plant growth, increased grain yield (151.6% compared to the control) and increased crude protein, WSP, and dry gluten content in seeds (65.3, 40.5, and 408.9% compared to the control, respectively). It also enhanced the grain mineral content; however it did not affect significantly on the grain starch content. Grain Phy-P content was increased by a higher level of NPK fertilization and interestingly, the level of phytase enzyme was also increased up to 46% in T_3 compared to the control. Moreover, a higher level of mother plant NPK fertilization has enhanced seed germination percentage, seedling fresh weight, phytase activity, Pi content in seeds, and Phy-P metabolism during the germination period, suggesting that grain nutritional quality can be improved by increasing NPK fertilization rates. The anti-nutritional compound Phy-P was also increased with increased NPK fertilizer levels; however, it may enhance seed viability, seed germination and seedling vigor.

Table 2.1 Effect of NPK fertilization on number of productive tillers per plant, number of grain per spike, 1000 grain weight, grain yield and percent increase in grain yield over control (T_1) in wheat. The same letter indicates no significant difference ($p < 0.05$).

Treatment	**No. of productive tillers**	**No. of grain**	**1000 grain weight**	**Grain yield**	**Percent increase in yield**
	(plant^{-1})	(spike^{-1})	(g)	(ton ha^{-1})	(%)
T_1	5.45^{c}	36.85^{b}	42.03^{b}	2.77^{c}	-
T_2	9.67^{b}	39.30^{ab}	46.29^{ab}	5.03^{b}	81.59^{b}
T_3	13.35^{a}	41.00^{a}	47.57^{a}	6.97^{a}	151.60^{a}

Table 2.2 Effect of NPK fertilization on grain mineral content of wheat. The same letter indicates no significant difference ($p < 0.05$).

Treatment	***N***	***P***	***Pi***	***K***	***Mg***	***Zn***	***Ca***
	(mg g^{-1})	(mg g^{-1})	(mg g $^{-1}$)	(mg g^{-1})	(mg g^{-1})	(µg g^{-1})	(µg g^{-1})
T_1	16.01^{c}	4.04^{c}	0.247^{b}	4.72^{b}	1.32^{c}	74.66^{b}	71.04^{b}
T_2	21.10^{b}	4.87^{b}	0.369^{a}	4.89^{ab}	1.43^{b}	87.97^{a}	107.89^{ab}
T_3	26.45^{a}	5.41^{a}	0.445^{a}	5.14^{a}	1.58^{a}	94.17^{a}	188.95^{a}

Table 2.3 Effect of NPK fertilization on grain quality of wheat. The same letter indicates no significant difference ($p < 0.05$).

Treatment	Starch	Crude protein	Total pentosan	Water soluble pentosan	Dry gluten	Phytate P
	(%)	(%)	(mg g^{-1})	(mg g^{-1})	(%)	(mg g^{-1})
T_1	65.08^a	8.87^c	8.58^a	1.11^b	3.7^c	3.14^c
T_2	62.98^a	11.69^{bc}	7.75^{ab}	1.34^{ab}	8.5^b	3.52^b
T_3	63.02^a	14.66^a	7.24^b	1.56^a	18.9^a	3.86^a

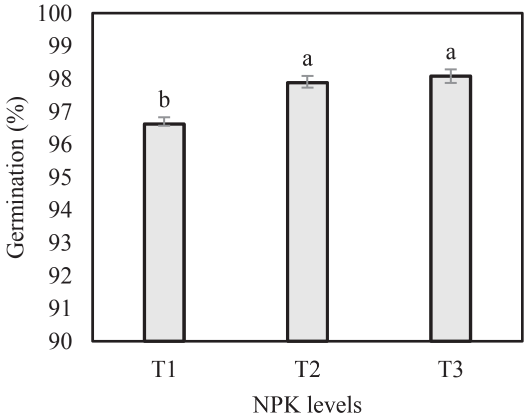

Fig. 2.1 Effect of mother plant NPK fertilization on seed germination percentage of wheat counted at 7 days from germination. The same letter indicates no significant difference ($p < 0.05$).

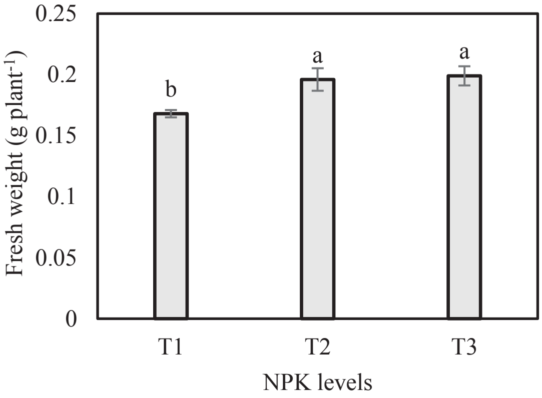

Fig. 2.2 Effect of mother plant NPK fertilization on seedling fresh weight of wheat at 7 days from germination. The same letter indicates no significant difference ($p < 0.05$).

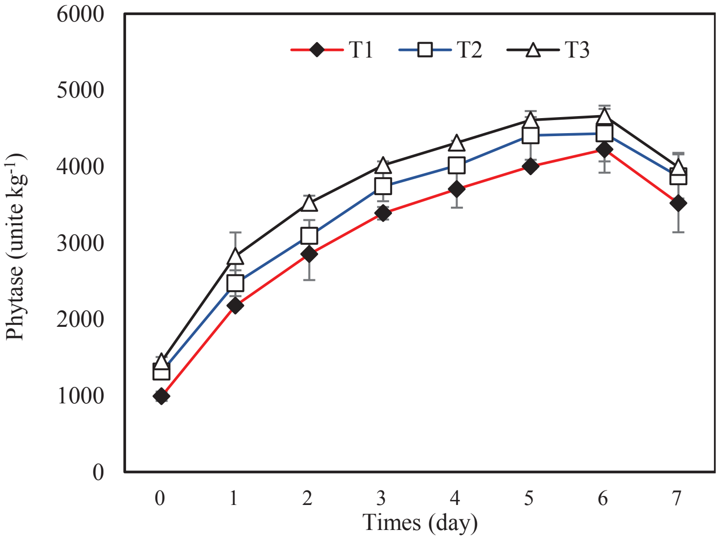

Fig. 2.3 Effect of mother plant NPK fertilization on phytase activity of wheat during seed germination.

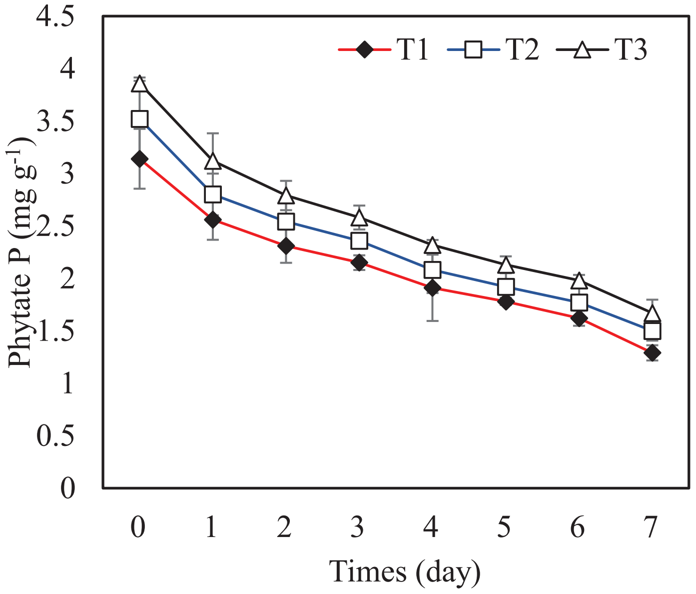

Fig. 2.4 Effect of mother plant NPK fertilization on Phy-P content of wheat during seed germination.

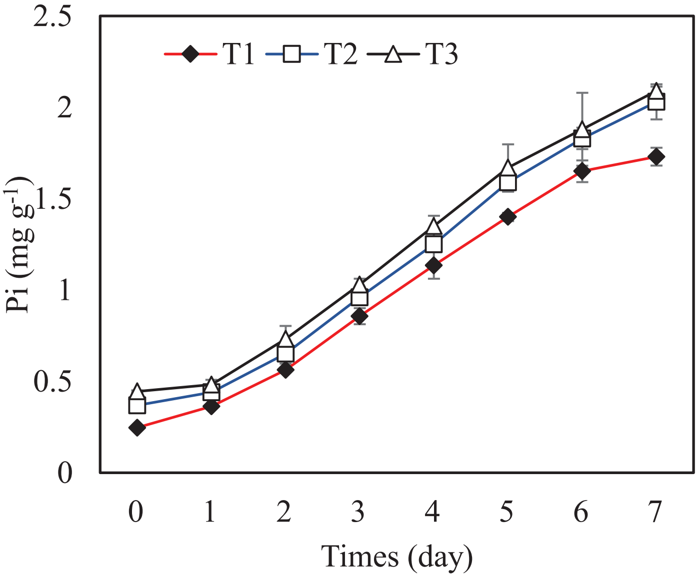

Fig. 2. 5 Effect of mother plant NPK fertilization on inorganic phosphorus (Pi) content of wheat during seed germination.

Chapter 3

Effect of drought stress and NPK fertilization on grain yield, nutritional and anti-nutritional quality of wheat

3.1 Introduction

Drought is a limiting factor that adversely affects wheat productivity and reduces plant growth by influencing several vital processes in plants, such as transpiration, translocation, mineral uptake and the metabolism of nutrients (Farooq *et al*. 2009). Crop production limitation rising from drought stress might be increased in the upcoming decades due to climatic change which may reduce precipitation and accelerate evapotranspiration (World Bank 2007; Lobell *et al.* 2008). Under drought stress condition, plant water potential and turgor decrease greatly, and therefore normal physiological functions cannot be performed (Zhu 2002). Drought stress affects adversely on leaf growth (Galle *et al*. 2010), leaf chlorophyll content (Brevedan and Egli 2007), stomatal conductance (Liang *et al.* 2002), and therefore it reduces photosynthetic activity and speed up leaf senescence (Yang and Zang 2006) which eventually results in declined growth and yield of crops (Ercoli *et al.* 2007). Plant survival or death under stress conditions, depends on severity of stress, duration and frequency of exposures, plant developmental stage, organ or tissues that expose to stress, and genetic characters (Larkindale *et al*. 2005; Farooq *et al*. 2009). Yang and Zang (2006) concluded that wheat is highly sensitive to drought stress during flowering and grain filling stages.

Increased doses of nitrogen (N) improved drought tolerance (Halvorson and Reule 1994). N fertilization mitigates the adverse effects of drought stress on plant growth by improving WUE (Saneoka *et al*. 2004). It also enhances leaf area and leaf chlorophyll content and thus improves photosynthetic efficiency and reduces photo-damage under drought stress condition (Wu *et al*. 2008). Phosphorus (P) fertilization effectively promotes plant growth under drought stress by enhancing root growth (Singh and Sale 1998), increasing water use efficiency (Garg *et al.* 2004; Waraich *et al*. 2011), increasing leaf area and photosynthetic activity (Singh *et al*. 2006), maintaining leaf stomatal conductance and nitrate reductase activity (Bruck *et al*. 2000; Naeem and Khan 2009) and increasing cell-membrane stability and water relations (Sawwan *et al*. 2000; Kang *et al.* 2014). Application of adequate level of potassium (K) enhances WUE (Egilla *et al*. 2005), photosynthetic activity, plant growth and yield in different crops under drought stress condition (Tiwari *et al.* 1998; Egilla *et al*. 2001). K fertilization alleviates detrimental effects of drought stress by improving many physiological processes such as regulation of turgor pressure and photosynthesis, enzymes activation and translocation of cations (Mengel and Kirkby 2001). Marschner (2012) concluded that K as an inorganic osmotica, plays a significant role in formation of the osmotic adjustment ability, even under drought condition. The effects of nutrients such as N, P, and K on enhancing plant growth, grain yield, and grain quality of plants under normal condition has been well studied so far; however, little is known about their function, if applied in combination, in improving grain yield, grain nutritional quality, pentosans content and Phy-P content of wheat under drought stress condition. Therefore, the present experiment was conducted with the aim to evaluate the effects of combined application of NPK on growth, productivity, grain mineral, starch, crude protein, TP, WSP, and grain Phy-P contents of wheat under drought stress condition.

3. 2 Materials and methods

3.2.1 Plant materials and growth conditions

This experiment was designed to evaluate the effect of combined fertilization of different levels of NPK on wheat productivity and grain nutritional quality under late season drought stress condition. A pot experiment was conducted in the greenhouse of the Faculty of Applied

Biological Sciences, Hiroshima University. Two wheat cultivars, Minaminokaori a commonly grown winter wheat in Japan and Lalmi-2 a facultative bread wheat grown in rainfed lands of Afghanistan were used in this study. The experiment was carried out in a vinyl greenhouse with natural sunlight and temperature to prevent nutrient leachate and avoid interruption by rainfall. Regosol, vermiculite (Asahi Kogyo Co. Ltd.), and nursery soil contained compost (Forex Torin Co. LTD. Japan) were mixed in a ratio of 2:1:1, and pots (10-liter capacity, top and bottom diameters of 29 and 25 cm and a depth of 27 cm) were filled with 10 kg. Chemical analysis of this mixture showed that it contained: 0.16 % N, 5.74 mg kg^{-1} available P, and 72.58 mg kg^{-1} available K. Furthermore, (6.4 g pot^{-1}) of dolomitic calcium magnesium carbonate was mixed with the soil to adjust the pH (H_2O) to 6.5. The present experiment was designed with 3 levels of NPK fertilizers: F_1 (150 kg N + 100 kg P_2O_5 + 75 kg K_2O ha^{-1}), F_2 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}), and F_3 (250 kg N + 140 kg P_2O_5 + 125 kg K_2O ha^{-1}). The sources of N, P and K were urea, single super phosphate (SSP), and potassium sulfate, respectively. They were applied in pots, as F_1 (2 g urea+3.76 g SSP+0.96 g potassium sulfate pot^{-1}), F_2 (2.8 g urea+4.51 g SSP+1.28 g potassium sulfate pot^{-1}) and F_3 (3.5 g urea+5.3 g SSP+1.6 g potassium sulfate pot^{-1}). All of P and K, and a half dose of N were applied before sowing, and the remaining N was applied in two equal splits at the time of tillering and anthesis stages. Wheat seeds were sown in the middle of November 2016, and then ten-day-old seedlings were transplanted in the pots (2 plants pot^{-1}), and the pots were arranged in a randomized complete block design with 4 replications. The plants were irrigated with tap water regularly until imposing the drought stress. Drought treatments consisting of well-irrigated, mild stress and severe stress were applied during the grain filling stage. The soil water content was adjusted to 20, 10 and 6% for well-irrigated, mild drought stress and severe drought stress conditions, respectively, and monitored by a Time Domain

Reflectometry (TDR-341F model, Fujiwara, Japan) digital moisture meter regularly. The drought stress continued for 40 days until the crop reached harvesting maturity. Plants were harvested at the end of May 2017, and the following parameters were recorded by the time of harvest and thereafter.

3.2.2 Growth, yield, and yield components

Number of fertile tillers (spike bearer tillers) per plant was counted before harvest. To measure 1000 kernel weight, 500 grains were counted, and weighted with a prescribed accuracy, and then the value was multiplied by 2. To determine the grain yield, mature spikes were collected, oven dried at 80°C for 48 hours, threshed, and the grain yield was recorded and expressed as grams per plant. The harvest index (HI) was calculated using the following formula:

$$\text{HI} = \frac{\text{Grain yield}}{\text{Total above ground biomass yield}} \text{ X } 100$$

3.2.3 Determination of grain mineral content

Samples of mature seeds were ground finely, and then digested by H_2SO_4/H_2O_2 (2:1, v/v) and diluted with distilled water. The content of total N, K, P, Ca, Mg and Zn was measured by following the methods and procedures described in page 12-13. Grain Pi content was extracted by overnight shaking, and the content was measured as explained in page 13.

. 3.2.4 Determination of grain starch and crude protein content

Grain starch content was measured following anthrone reagent method and grain crude protein content was calculated using the total N value which was obtained by Kjeldahl method (Jones 1991) as explained in page 13.

3.2.5 Determination of grain TP, WSP and Phy-P content

Grain TP and WSP were measured using $FeCl_3$-orcinol reagents, and grain Phy-P content was extracted and measured following Raboy and Dickinson (1984) method (as described in page 13-14).

3.2.6 Statistical analysis

The data were subjected to three-way analysis of variance in CoStat software (CoStat ver. 6.45; CoHort Software) to examine the impact of drought stress (well-irrigated, mild stress and severe-stress), wheat cultivar (Minaminokaori and Lalmi-2), NPK fertilization (F_1, F_2 and F_3) and their interactions on response variables. Significant differences between means were assessed using Duncan Multiple Range Test at the probability level of 0.05.

3.3 Results

3.3.1 Grain yield and yield components

Statistical analysis of data showed that drought stress significantly reduced the number of fertile tillers, 1000 kernel weight, grain yield and harvest index in both cultivars (Table 3.1). Application of a high rate of NPK (F_3) resulted in the production of a higher number of fertile tillers in both cultivars under well-irrigated condition, while F_1 treatment recorded the lowest number of fertile tiller in Minaminokaori cultivar under severe drought stress condition. F_3 treatment enhanced the production of fertile tillers in both cultivars under mild and severe stress conditions. Cultivar Lalmi-2 recorded higher number of fertile tillers under all levels of NPK and drought stress treatments compared to that of Minaminokaori.

The 1000 kernel weight was significantly different between cultivars, NPK treatment levels and drought stress levels. Cultivar Lalmi-2 recorded higher 1000 kernel weight under all levels of NPK fertilization and drought stress conditions compared to Minaminokaori. NPK treatments slightly increased 1000 kernel weight in Minaminokaori under all levels of drought stress levels however, the mean values did not differ significantly. F_3 and F_2 treatments recorded the highest 1000 kernel weight in Lalmi-2 cultivar under well-irrigated condition. NPK fertilization significantly increased 1000 kernel weight in Lalmi-2 under all levels of stress conditions. In comparison with F_1, F_3 treatment increased 1000 kernel weight in Lalmi-2 cultivar by 7.7 and 16.8 % under mild stress and severe-stress conditions respectively. In Minaminokaori, increase in 1000 kernel weight due to the high rate of NPK (F_3) fertilization was 9.1% under mild stress and 4.8% under severe stress compared to F_1 treatment.

Grain yield was negatively affected by drought stress in both cultivars. Lalmi-2 recorded higher grain yield under all levels of drought stress conditions compared to Minaminokaori. NPK treatment significantly increased grain yield. F_3 treatment recorded higher grain yield in both cultivars under drought stress condition. It was observed that mild drought stress had less effect on grain yield of Lalmi-2 cultivar. F_3 treatment recorded the highest grain yield in Lalmi-2 under well-irrigated and mild stress conditions. Grain yield was the lowest in cultivar Minaminokaori under severe drought stress condition with the lowest NPK fertilizer (F_1) level. F_3 treatment increased grain yield by 38.2% under mild stress and 24.0% under severe-stress conditions compared to F_1 treatment in Lalmi-2 cultivar. However, in Minaminokaori increase in grain yield due to high NPK (F_3) fertilization under mild and severe stress conditions compared to F_1 treatment was 29.9 and 35.0% respectively.

Harvest index was significantly reduced in Minaminokaori cultivar under drought stress condition however, in Lalmi-2 a higher harvest index was observed under mild stress condition. F_3 treatment increased harvest index in both cultivars. The highest harvest index was recorded in Lalmi-2 cultivar under mild stress condition where a high rate of NPK (F_3) was also applied. However, F_1 treatment recoded the lowest harvest index in Minaminokaori cultivar under all levels of stress conditions. In comparison with F_1, F_3 treatment increased harvest index by 14.9% under mild stress condition and 8.7% under sever-stress condition in Lalmi-2 cultivar. In Minaminokaori cultivar F_3 treatment increased the harvest index compared to F_1 treatment by 5.2 and 9.2% under mild and severe stress conditions, respectively.

3.3.2 Grain mineral content

Mineral content was highly affected by drought stress and NPK treatments (Table 3.2). Drought stress increased total N content in both cultivars. Minaminokaori recorded a higher total N content compared to Lalmi-2. NPK fertilization enhanced total N content in both cultivars. F_3 treatment recorded the highest total N content in Minaminokaori under severe-stress condition. The lowest total N content was recorded in Lalmi-2 cultivar under well-irrigated condition where the lowest rate of NPK (F_1) was applied. Grain K content was also increased under drought stress condition. The highest value of grain K content was observed under severe drought stress condition in F_3 treatment of cultivar Lalmi-2. NPK treatment enhanced total K content in both cultivars, however the response was greater in cultivar Lalmi-2. The lowest K content was observed in Minaminokaori cultivar under well-irrigated condition with the lowest rate of NPK (F_1) treatment. Grain total P content was recorded higher in Minaminokaori cultivar under severe drought stress condition. Grain total P content was greater with F_3 treatment under well-irrigated as well as drought stress conditions. Minaminokaori recorded higher total P content under mild and sever stress conditions than Lalmi-2. F_1 treatment recorded the lower total P content in both cultivars under well-irrigated condition. Drought stress increased Pi content in Minaminokaori cultivar. Pi content was reduced in Lalmi-2 cultivar due to severe stress condition. The highest value of grain Pi content was recorded in Minaminokaori cultivar under severe drought stress condition with high rate of NPK fertilizer application. F_1 treatment in Lalmi-2 cultivar recorded the lowest Pi content under severe stress condition. NPK fertilization enhanced Mg content and the highest Mg content was observed in Minaminokaori cultivar with F_3 treatment under sever condition. The lowest Mg content was observed in well irrigated Lalmi-2 with low NPK (F_1) application. Minaminokaori recorded a higher Mg content under all levels of stress conditions compared to Lalmi-2. NPK fertilization enhanced Ca content and the highest value of Ca was recorded with F_3 treatment in Minaminokaori under mild-stress condition. The lowest Ca content was observed in Lalmi-2 cultivar under severe-stress condition with the lowest level of NPK (F_1) application. Minaminokaori recorded a higher Ca content irrespective of NPK treatments and drought stress condition compared to Lalmi-2. Zn content was found to be higher in Minaminokaori cultivar irrespective of drought stress and NPK levels compared to Lalmi-2. F_3 treatment slightly enhanced Zn content in both cultivars but the mean values did not differ significantly within the same cultivar. F_3 treatment increased N, K, P, Pi, Mg, Ca and Zn contents in grain by 9.1, 6.4, 9.8, 9.4, 5.2, 26.4 and 14.6% compared to F1 treatment under mild stress, respectively, and by 10.1, 5.1, 3.1, 36.4, 3.0, 13.2 and 7.5%, respectively under severe stress condition in Minaminokaori. However, in cultivar Lalmi-2, the high level of NPK fertilization (F_3) increased N, K, P, Pi, Mg, Ca and Zn content in grain by 5.1, 3.6, 6.3, 33.5, 5.1, 8.4 and 8.5%, respectively under mild stress and by 15.2, 4.1, 7.0, 31.0, 1.7, 15.3 and 6.4%, respectively under severe stress condition**.**

3.3.3 Grain starch content

Grain starch content decreased by drought stress (Table 3.3). The highest grain starch content was recorded in cultivar Lalmi-2 under well-irrigated condition, while the lowest was observed in Minaminokaori under severe stress condition. Grain starch content was decreased with increase in NPK rate. In comparison with well-irrigated condition, mild and severe drought stress decreased grain starch content by 1.5 and 2.6%, respectively in cultivar Minaminokaori, and by 2.7 and 3.4% respectively in cultivar Lalmi-2.

3.3.4 Grain crude protein content

Grain crude protein content was increased under drought stress condition, and the higher crude protein was observed under severe-stress condition. Minaminokaori recorded higher crude protein content compared to Lalmi-2 irrespective of NPK levels and drought stress condition (Table 3.3). NPK fertilization significantly enhanced crude protein content in both cultivars, and F_3 treatment recorded the highest crude protein content in Minaminokaori under severe-stress condition. However, the lowest crude protein content was recorded in Lalmi-2 cultivar under well-irrigated condition where the lowest rate of NPK (F_1) was applied. In comparison to F_1, F_3 treatment increased grain crude protein in cultivar Minaminokaori by 9.1 and 10.1% under mild and severe stress conditions respectively. For the cultivar Lalmi-2 the increase due to high NPK (F_3) fertilization was 4.8 and 15.2% under mild and severe stress conditions, respectively.

3.3.5 Grain TP and WSP content

Grain TP content was recorded higher under mild stress condition in both cultivars. Increased levels of NPK (F_3) reduced TP content, while low NPK (F_1) treatment under mild stress condition resulted in the highest grain TP content in cultivar Minaminokaori. The lowest TP content was observed in cultivar Lalmi-2 under severe-stress condition where a high level of NPK (F_3) was also applied (Table 3.3). Grain WSP content was negatively affected by drought stress condition. Different from TP content, WSP content was recorded higher under well-irrigated condition. F_3 treatment resulted in the highest grain WSP content in cultivar Minaminokaori under well-irrigated condition. NPK fertilization enhanced WSP content and F_3 treatment recorded higher WSP under both levels of drought stress conditions in both cultivars (Table 3.3). Cultivar Minaminokaori recorded a higher WSP content under will-irrigated as well as drought stress conditions. Compared to F_1, F_3 treatment increased grain WSP content in Minaminokaori by 9.7, 14.0 and 8.7 under well-irrigated, mild stress and severe stress conditions, respectively. These values for the cultivar Lalmi-2 were 11.3, 13.4 and 22.5%.

3.3.6 Grain Phy-P content

Drought stress significantly increased grain Phy-P content and the highest Phy-P content was recorded under severe stress condition. NPK fertilization enhanced the accumulation of Phy-P in both cultivars under well-irrigated as well as drought stress conditions (Table 3.3). Cultivar Minaminokaori recoded a higher Phy-P content under severe-stress condition than cultivar Lalmi-2. F_3 treatment recorded the highest Phy-P content in Minaminokaori cultivar under severe-stress condition. However, F_1 treatment resulted in lower level of accumulation of Phy-P in both cultivars under will-irrigated condition. F_3 treatment increased grain Phy-P content in Minaminokaori cultivar by 11.0, 8.9 and 4.1% under well-irrigated, mild stress and severe stress conditions respectively, compared to F_1 treatment. These values for the cultivar Lalmi-2 were 3.4, 3.8 and 3.5%. Severe drought stress increased grain Phy-P content by 10.7 % in Minaminokaori and by 11.3 % in Lalmi-2, compared to well-irrigated condition.

3.4 Discussion

The results of this study indicated that drought stress adversely affected grain yield and yield components irrespective of NPK treatment levels in both cultivars. Higher levels of combined NPK fertilization improved wheat performance under drought stress condition by enhancing plant productivity, grain mineral content, and grain quality. A high rate of NPK (F_3) fertilization improved photosynthetic activity by enhancing water and nutrient absorption that resulted in producing more assimilates. As a result, increase in number of fertile tillers, 1000 kernel weight, grain yield and harvest index were observed under well-irrigated as well as drought stress conditions. Cultivar Lalmi-2 was more responsive to NPK fertilization and therefore it exhibited greater number of spikes per plant, higher 1000 kernel weight, higher grain yield and increased harvest index under both well-irrigated and drought stress conditions. This result could be contributed to the genetic potential of Lalmi-2 compared to Minaminokaori. Ati *et al*. (2016) concluded that application of NPK fertilizer increased number of spikes and grain yield of wheat under drought stress condition. Akram *et al*. (2014) found that application of a higher rate of N improves water use efficiency and enables the plants to survive and thus produce high grain yield under drought stress condition. Increase in grain yield and yield components of wheat through NPK fertilization under well-watered condition was reported by many researchers (Hussain *et al*. 2002; Laghari *et al.* 2010; Abdel-Aziz *et al*. 2016), however there is insufficient information on effects of combined application of NPK on wheat yield and yield components under environmental stress conditions including drought.

The grain mineral content was significantly influenced by drought stress as well as the level of NPK fertilization and the cultivar differences. It was observed that content of N, K, P, Pi, Mg and Ca in grain were increased under drought stress condition. Content of mineral in grains were significantly increased with an increase in NPK rate with an exception of Zn. Drought stress induced leaf senescence (Sarwat *et al*. 2013), and therefore mineral might be transported from leaves to the meristematic tissues and developing grains. The increase in N content in plants under drought stress is due to accumulation of free amino acids that are not synthesized into proteins (Alam 1999). Under drought stress condition, most of the N and P was accumulated in grains (Patel and Singh 1998). F_3 treatment in our study consisted of a high rate of N, P and K fertilizers that enhanced N, P, K content in wheat grains. Raza *et al*. (2013) reported that application of a high dose of K, improved K, N, P and Ca uptake of wheat under drought stress condition. In this study, it was found that grain Pi content was increased under drought stress condition. The effect of drought stress and NPK fertilization on grain Pi content was not sufficiently reported, however a positive relationship was observed between total P and Pi content in this study, which could be a reason for a high Pi content. P uptake is enhanced when the dose of P fertilizer was increased (Bolland *et al*. 1999; Saha *et al*. 2014). Grain Mg content was higher in Minaminokaori cultivar under well-irrigated and mild stress conditions, however it was lower under severe drought stress condition. F_3 treatment recorded a higher Mg content under well-irrigated and mild stress conditions in both wheat cultivars. Slamka *et al*. (2011) concluded that N fertilization exhibits more expressive effect on plant Mg uptake under optimal water supply condition. Cultivar

Minaminokaori recorded higher Zn content compared to Lalmi-2 irrespective of drought stress and NPK treatments. It could be assumed that Lalmi-2 produced a higher grain yield compared to Minaminokaori and thus grain Zn content was decreased in Lalmi-2.

Drought stress significantly reduced grain starch content in both cultivars. This reduction was due to the adverse effects of drought stress that decreased plant photosynthetic activity, and therefore carbohydrate formation and accumulation of starch was declined. Drought stress during anthesis and grain filling remarkably decreases starch content of wheat (Saeedipour 2011). The starch content was decreased with increase in the rate of NPK fertilization. Lalmi-2 cultivar recorded higher starch content and low crude protein content compared to Minaminokaori. Kindred *et al.* (2008) observed a negative relationship between crude protein and starch content, and N fertilization decreased the starch content of wheat grain. Hlisnikovsky and Kunzova (2014) also found a higher grain starch content in those plants which no fertilizer was supplied. In this study grain crude protein content was enhanced under drought stress condition, and an elevated level of NPK fertilization greatly increased the crude protein content under both well-irrigated and drought stress conditions. Under well- irrigated condition, grain protein content may decrease by dilution of N with carbohydrates (Guttieri *et al*. 2005). Singh *et al*. (2012) also observed a significant increase in protein content of wheat grown under rainfed condition. NPK fertilization increased the synthesis of the raw protein in wheat (Crista 2012). A high level of NPK fertilization enhanced grain crude protein content in the wheat variety Inqulab 91 (Sameen *et al*. 2002). In this study, F_3 treatment consisted a high level of N and K fertilizers that resulted in a higher crude protein content. Many researchers have found that adequate amount of K fertilization improves plant N uptake and protein synthesis (Alam *et al*. 2009; Lakudzala 2013; Daniel *et* *al*. 2016). Lalmi-2 recorded a lower grain crude protein content compared to Minaminokaori, and it might be due to the higher yield capacity of Lalmi-2 cultivar and varietal difference in uptake of N and protein synthesis.

Grain TP content was increased under mild drought stress condition. However, a higher level of NPK fertilization slightly decreased TP content in both cultivars under both levels of drought stress conditions. It was assumed that under drought stress, plants produced less grain yield, and most of the non-starch polysaccharides were accumulated in these grains. Effect of NPK fertilization on grain TP and WSP under drought stress condition in wheat, has not been reported sufficiently by earlier research studies. The result of this investigation indicated that WSP content was reduced under drought stress condition and NPK fertilization significantly increased WSP content under both well-irrigated and drought stress conditions. Water-unextractable pentosan had a negative effect, while WSP had a positive impact on the bread-making quality of wheat (Courtin and Delcour 2002).

Phy-P serves as seed storage for P, but this compound can contribute to a nutritional problem, since it binds with proteins and some important micronutrients, such as Fe and Zn, and significantly reduces their availability (Rosa 1999; Raboy 2001). In this study, grain Phy-P content was increased with severity of drought and increased rate of NPK fertilization. The effect of NPK fertilization on the Phy-P content of wheat grain under drought stress condition was not sufficiently reported by other researchers so far. Phy-P is the major storage form of P in cereals, therefore the content of this antinutrient compound mostly depends on the grain total P content. Application of a high rate of P fertilizer may results in a high phytate content (Raboy and Dickinson 1984). The reason for a higher Phy-P synthesis and accumulation in wheat grains in F_3 treatment may be contributed to the higher rate of P fertilizer application.

3.5 Summary

In this chapter, the possibility of improving productivity and grain nutritional quality of wheat by combined application of N, P and K fertilizers under drought stress condition was investigated. The result indicated that drought stress significantly decreased grain yield, grain starch content, and WSP content, but increased grain crude protein content, TP content, and Phy-P content of both cultivars. Between the cultivars, Lalmi-2 exhibited drought tolerance, and recorded higher grain yield, total K and starch content under both well-irrigated and drought stress conditions, while Minaminokaori recorded higher grain total N, P, Ca, Mg, Zn, Pi, crude protein, TP, WSP and Phy-P content. Therefore, further increase in NPK rate can ameliorate the adverse effects of drought stress, and enhances plant productivity, grain mineral, crude protein and WSP content under drought stress condition. Besides applying a higher rate of NPK fertilizers, it is beneficial to use a fertilizer responsive and drought-tolerant genotype such as Lalmi-2 to minimize the risk of yield loss due to drought stress.

Table 3.1 Effect of NPK fertilization on number of fertile tillers, 1000 kernel weight, grain yield and harvest index of wheat under drought stress condition. Means with different letters are significantly different from each other at $p < 0.05$.

Cultivars	Drought stress levels	NPK levels	No. Fertile tillers (plant^{-1})	1000 Kernel weight (g)	Grain yield (g plant^{-1})	Harvest index
Minaminokaori	Well-irrigated	F_1	19.50^{fg}	39.79^{f}	33.79^{gh}	38.20^{f}
		F_2	22.75^{abcde}	39.89^{f}	42.34^{e}	40.46^{def}
		F_3	25.25^{ab}	40.44^{f}	47.11^{d}	41.30^{cde}
	Mild stress	F_1	19.75^{efg}	36.45^{f}	31.61^{hi}	39.03^{ef}
		F_2	23.50^{abcd}	37.41^{f}	40.62^{ef}	40.36^{def}
		F_3	25.25^{ab}	39.78^{f}	41.05^{ef}	41.05^{cde}
	Severe stress	F_1	18.00^{g}	36.61^{f}	27.52^{i}	38.33^{f}
		F_2	20.75^{defg}	37.09^{f}	31.99^{hi}	40.45^{def}
		F_3	22.50^{bcdef}	38.35^{f}	36.97^{fg}	41.86^{cd}
Lalmi-2	Well-irrigated	F_1	21.25^{cdef}	55.40^{abc}	50.16^{d}	39.78^{def}
		F_2	24.00^{abc}	56.96^{a}	63.04^{b}	42.01^{cd}
		F_3	24.75^{ab}	57.79^{a}	69.68^{a}	44.49^{b}
	Mild stress	F_1	20.75^{defg}	52.09^{cd}	50.33^{d}	41.04^{cde}
		F_2	23.00^{abcd}	52.53^{bcd}	57.46^{c}	43.53^{bc}
		F_3	25.75^{a}	56.11^{ab}	69.55^{a}	47.17^{a}
	Severe stress	F_1	20.75^{defg}	47.50^{e}	48.45^{d}	41.64^{cde}
		F_2	22.50^{bcdef}	50.21^{de}	56.42^{c}	45.16^{ab}
		F_3	23.75^{abcd}	55.50^{abc}	60.07^{bc}	45.27^{ab}

Table 3.2 Effect of NPK fertilization on mineral contents of wheat grains under drought stress condition. Means with different letters are significantly different from each other at $p < 0.05$.

Cultivars	**Drought stress levels**	**NPK levels**	**N (mg g^{-1})**	**K (mg g^{-1})**	**P (mg g^{-1})**	**Pi (mg g^{-1})**	**Mg (mg g^{-1})**	**Ca (µg g^{-1})**	**Zn (µg g^{-1})**
Minaminokaori	Well-irrigated	F_1	23.95^{ef}	5.23^{g}	3.91^{e}	0.174^{bcde}	1.22^{cdefgh}	177.69^{i}	64.41^{a}
		F_2	25.29^{de}	5.31^{fg}	4.18^{bcde}	0.181^{bcde}	$1.31^{abcdefg}$	286.98^{def}	65.87^{a}
		F_3	26.40^{cd}	5.51^{efg}	4.27^{abcd}	0.185^{bcde}	1.40^{a}	338.65^{cd}	66.62^{a}
	Mild stress	F_1	24.94^{de}	5.28^{g}	4.17^{bcde}	0.180^{bcde}	1.34^{abcde}	335.20^{cde}	64.04^{a}
		F_2	26.19^{cd}	5.53^{efg}	4.37^{abcd}	0.190^{bcde}	1.39^{ab}	394.73^{abc}	65.71^{a}
		F_3	27.22^{bc}	5.62^{efg}	4.58^{ab}	0.197^{bcd}	1.41^{a}	423.60^{a}	73.39^{a}
	Severe stress	F_1	26.36^{cd}	5.34^{fg}	4.52^{ab}	0.184^{bcde}	1.32^{abdef}	365.45^{abc}	61.92^{a}
		F_2	28.54^{ab}	5.55^{efg}	4.53^{abc}	0.192^{bcde}	1.35^{abcde}	409.93^{ab}	62.50^{a}
		F_3	29.01^{a}	5.61^{efg}	4.66^{a}	0.251^{a}	1.36^{abc}	413.85^{ab}	66.56^{a}
Lalmi-2	Well-irrigated	F_1	20.48^{h}	5.79^{def}	3.90^{e}	0.160^{de}	1.11^{h}	258.82^{fgh}	39.16^{b}
		F_2	21.10^{gh}	5.94^{cde}	4.02^{de}	0.214^{abc}	1.17^{fgh}	271.28^{efg}	48.41^{b}
		F_3	21.53^{gh}	6.20^{bcd}	4.13^{cde}	0.221^{ab}	1.19^{efgh}	353.47^{bc}	49.01^{b}
	Mild stress	F_1	21.41^{gh}	6.34^{bc}	3.97^{de}	0.152^{de}	1.18^{efgh}	209.59^{ghi}	38.71^{b}
		F_2	21.49^{gh}	6.51^{ab}	4.13^{cde}	0.158^{de}	1.20^{defgh}	216.26^{ghi}	41.32^{b}
		F_3	22.51^{fg}	6.57^{ab}	4.22^{bcde}	0.203^{bcd}	$1.24^{bcdefgh}$	227.09^{fghi}	42.00^{b}
	Severe stress	F_1	21.64^{gh}	6.57^{ab}	4.28^{abcd}	0.145^{e}	1.15^{gh}	187.74^{i}	44.32^{b}
		F_2	22.27^{g}	6.61^{ab}	4.51^{abc}	0.169^{cde}	1.16^{efgh}	196.49^{hi}	46.11^{b}
		F_3	24.93^{de}	6.84^{a}	4.58^{ab}	0.190^{bcde}	1.17^{fgh}	216.48^{ghi}	47.17^{b}

Table 3.3 Effect of NPK fertilization on grain starch, crude protein, TP, WSP, and Phy-P content of wheat under drought stress condition. Means with different letters are significantly different from each other at $p < 0.05$.

Cultivars	**Drought stress levels**	**NPK levels**	**Starch (%)**	**Crude protein (%)**	**TP (mg g^{-1})**	**WSP (mg g^{-1})**	**Phy-P (mg g^{-1})**
Minaminokaori	Well-irrigated	F_1	66.76^{bcde}	13.26^{ef}	8.02^{ab}	1.85^{abc}	2.72^{bc}
		F_2	66.42^{bcde}	14.01^{de}	7.92^{ab}	1.93^{ab}	2.97^{abc}
		F_3	65.34^{cdef}	14.63^{cd}	7.88^{ab}	2.03^{a}	3.02^{abc}
	Mild stress	F_1	66.37^{bcde}	13.82^{de}	8.37^{a}	1.64^{abcd}	2.93^{abc}
		F_2	65.13^{def}	14.51^{cd}	8.30^{ab}	1.75^{abcd}	3.07^{abc}
		F_3	63.95^{ef}	15.08^{bc}	8.09^{ab}	1.87^{abc}	3.19^{abc}
	Severe stress	F_1	65.86^{cdef}	14.60^{cd}	8.17^{ab}	1.49^{cd}	3.16^{abc}
		F_2	65.34^{cdef}	15.81^{ab}	7.87^{ab}	1.62^{abcd}	3.19^{abc}
		F_3	62.20^{f}	16.07^{a}	7.69^{ab}	1.76^{abcd}	3.29^{a}
Lalmi-2	Well-irrigated	F_1	71.23^{a}	11.34^{h}	7.95^{ab}	1.68^{abcd}	2.71^{c}
		F_2	70.17^{ab}	11.69^{gh}	7.87^{ab}	1.79^{abcd}	2.84^{abc}
		F_3	69.55^{abc}	11.93^{gh}	7.84^{ab}	1.87^{abc}	2.91^{abc}
	Mild stress	F_1	69.13^{abcd}	11.86^{gh}	8.29^{ab}	1.57^{bcd}	2.87^{abc}
		F_2	68.45^{abcd}	11.90^{gh}	8.21^{ab}	1.68^{abcd}	2.96^{abc}
		F_3	67.63^{abcde}	12.47^{fg}	8.05^{ab}	1.78^{abcd}	2.98^{abc}
	Severe stress	F_1	68.49^{abcd}	11.99^{gh}	8.12^{ab}	1.38^{d}	3.11^{abc}
		F_2	67.79^{abcde}	12.34^{g}	7.85^{ab}	1.59^{bcd}	3.16^{abc}
		F_3	67.51^{abcde}	13.81^{de}	7.66^{b}	1.69^{abcd}	3.22^{ab}

Table 3.4 Significance of different source of variance.

Parameters	**Source of variation**						
	DS	**C**	**NPK**	**DS x C**	**DS x NPK**	**C x NPK**	**DS x C x NPK**
No. of fertile tillers (plant^{-1})	0.027	0.099	<0.001	0.506	0.943	0.618	0.630
1000 kernel weight (g)	<0.001	<0.001	<0.001	0.190	0.135	0.781	0.850
Grain yield (g plant^{-1})	<0.001	<0.001	<0.001	0.055	0.044	0.007	0.049
Harvest index	0.0703	<0.001	<0.001	0.038	0.374	0.047	0.051
Grain N content (mg g^{-1} DW)	<0.001	<0.001	<0.001	0.340	0.191	0.247	0.909
Grain K content (mg g^{-1} DW)	<0.001	<0.001	0.008	0.049	0.936	0.998	0.853
Grain P content (mg g^{-1} DW)	<0.001	0.017	0.001	0.426	0.992	0.716	0.913
Grain Pi content (mg g^{-1} DW)	0.646	0.439	0.003	0.068	0.918	0.168	0.552
Grain Mg content (mg g^{-1} DW)	0.062	<0.001	0.018	0.876	0.857	0.674	0.801
Grain Ca content (mg g^{-1} DW)	0.158	<0.001	<0.001	<0.001	0.172	0.034	0.688
Grain Zn content (mg g^{-1} DW)	0.769	<0.001	0.101	0.289	0.923	0.717	0.488
Grain Starch content (% DW)	0.033	<0.001	0.165	0.838	0.998	0.973	0.929
Grain crude protein content (% DW)	<0.001	<0.001	<0.001	0.341	0.187	0.240	0.906
Grain TP content (mg g^{-1} DW)	0.013	0.555	0.163	0.997	0.897	0.992	0.997
Grain WSP content (mg g^{-1} DW)	0. 005	0.122	0.020	0.781	0.986	0.992	0.995
Grain Phy-P content (mg g^{-1} DW)	0.003	0.204	0.091	0.923	0.994	0.831	0.957

Probability values in the table are related with three-way analysis of variance for factors drought stress (DS), cultivar (C), NPK, and the interaction of drought stress x cultivar (DS x C), drought stress x NPK (DS x NPK), cultivar x NPK (C x NPK) and drought stress x cultivar x NPK (DS x C x NPK). (Significance at $p < 0.05$)

Chapter 4

Effect of combined salicylic acid and potassium application on grain yield, nutritional and anti-nutritional quality of wheat under drought stress condition

4.1 Introduction

Drought stress negatively affects plant growth and productivity and threatens successful crop production more than other environmental stresses (Zhu 2002). Approximately 32 % of wheat fields suffer from several types of drought stresses throughout their growth period in developing countries (Morris *et al.* 1991). Drought stress at anthesis stage decreases pollination and therefore, number of grains per spike and grain yield is reduced (Ashraf 1998).

Potassium (K) can play effective roles in the physiological processes of photosynthesis, formation of carbohydrates and protein, transportation of water and nutrients, nitrogen (N) utilization, and stimulation of early growth in plants (Daniel *et al*. 2016; Lakudzala 2013). It controls stomata opening and enhances enzyme activation in plants, therefore it improves crop productivity (Yawson *et al*. 2011). Application of K enhances transportation of water, nutrients, and carbohydrates in plant tissue (De La Guardia and Benlloch 1980). Under drought stress condition, leaf stomata fail to function actively in K-deficient plants, and subsequently, cause excessive water loss (Egilla *et al*. 2005).

Salicylic acid (SA; 2-hydroxybenzoic acid) is a phenolic phytohormone which enhances plant defense against various biotic and abiotic stresses through morphological, biochemical, and physiological mechanisms (War *et al*. 2011). SA alleviates the detrimental effects of different abiotic stresses by enhancing the internal level of various hormones in plants (Sakhabutdinova *et al*. 2003). Waseem *et al*. (2006) and Arfan *et al*. (2007) reported that SA is a conservative compound of some biotic and abiotic stresses and it functions primarily as a molecular signal for the adjustment of plants under abiotic stress conditions.

According to Gunes *et al*. (2005), plant growth, transpiration rates, stomatal regulation and photosynthetic processes, ion uptake, and transport, are regulated by SA.

Pentosan is a major fiber component of the non-starch polysaccharides in cereal, commonly referred to as flour gum or hemicellulose. Pentosan is an important component of bread dough in which they bind water and contribute to the formation of viscous dough (Buksa *et al*. 2010). WSP has a positive effect on the bread-making quality of wheat flour (Courtin and Delcour 2002). Unlike pentosan, phytate is known as an anti-nutritional factor, because it binds with proteins and some important micronutrients, such as Fe and Zn, and significantly reduces their availability (Raboy 2001). Although the adverse effects of drought on the content of these compounds has been studied (Rakszegi *et al*. 2014; Patel and Singh 1998), the mechanism of improvement of these components under drought stress condition has not been examined. The aim of this chapter was to investigate whether productivity and grain quality of wheat can be improved by combined effect of K and SA application. Therefore, the present experiment was designed to evaluate the combined effects of SA and K on productivity, grain mineral, starch, crude protein, TP, WSP, and Phy-P content of wheat under drought stress condition.

4.2 Materials and methods

4.2.1 Plant material and growth conditions

This study was conducted in a vinyl greenhouse in the Faculty of Applied Biological Sciences, Hiroshima University with natural sunlight and temperature. A commonly grown wheat cultivar in Japan, Minaminokaori, was used in this study. Regosol, vermiculite (Asahi Kogyo Co. Ltd.), and nursery soil contained compost (Forex Torin Co. LTD. Japan) were mixed in a ratio of 2:1:1, and pots (9-liter capacity, 25 cm at the top and 23 cm at the bottom in diameter, and 23 cm in depth) were filled with 8.25 kg. The chemical composition of this mixture was: 0.14 % total N, 4.85 mg kg^{-1} available P, and 65.73 mg kg^{-1} available K. Meanwhile dolomitic calcium magnesium carbonite (4.9 g pot^{-1}) was mixed with the soil to adjust the pH (H_2O) to 6.5.

K treatment comprised of 3 levels (50, 100 and 200 kg ha^{-1} potassium sulfate). They were applied in pots as, K_1 (0.49g pot^{-1}), K_2 (0.98 g pot^{-1}), and K_3 (1.96 g pot^{-1}). All 3 doses of K fertilizer and 4.32 g pot^{-1} of single super-phosphate were applied to the soil after pot preparation. N fertilization (2.13 g urea pot^{-1}) was carried out in 3 split doses, half dose of urea was applied prior to transplanting, and the remaining second and third splits of urea were applied at tillering and anthesis stages, respectively. Wheat seeds were sown in the middle of November 2016, and then 10-day-old seedlings were transplanted into the pots (one plant per pot) and arranged in a randomized complete block design with 4 replicates. The plants were irrigated regularly with the tap water.

SA treatment was carried out at the heading stage for both will-irrigated and drought stressed plants. To compare the effect of SA treatment with those of non-treated plants, one set of plants was foliar sprayed with 0.7 mM salicylic acid (2 ml per plant), while another set of plants was sprayed with 2 ml of distilled water per plant (SA-untreated).

Drought treatments consisting of well-irrigated and drought stress conditions were imposed at the anthesis stage, by adjusting the soil water content to 18.0% and 9.0% for the well-irrigated, and drought stress conditions, respectively, using Time Domain Reflectometry (TDR-341F model, Fujiwara, Japan) digital moisture meter. The moisture content was regularly monitored with the TDR.

4.2.2 Grain yield and yield components

Wheat plants were harvested at the end of May 2017, when all spikes were fully matured. The number of fertile tillers was counted in each plant by the time of harvest and expressed as the number of fertile tillers per plant. To measure 1000 kernel weight, 500 grains were counted and weighted with a prescribed accuracy, then the value was multiplied by 2. Grain yield was recorded per plant and expressed in g plant^{-1}.

4.2.3 Determination of grain mineral content

Samples of mature seeds were ground finely, and then digested by H_2SO_4/H_2O_2 (2:1, v/v) and diluted with distilled water. The content of total N, K, P, Ca, Mg and Zn was measured following the methods and procedures described in page 12-13. Grain Pi content was extracted by overnight shaking, and the content was measured as explained in page 13.

4.2.4 Determination of grain starch, protein, TP and WSP content

The starch content of wheat grains was determined following anthrone reagent method (Nag 2016), and crude protein content was calculated using total N content value obtained by Kjeldahl method (as explained in page 13). Grain TP and WSP content was measured following the orcinol-HCl method as suggested by (Hashimoto *et al.* 1986). The details for extraction and measurement of TPz and WSP content is provided in page 13-14.

4.2.5 Determination of grain Phy-P

Grain Phy-P content was extracted and assayed for P using ammonium molybdate reaction reagent as explained in page 14.

4.2.6 Statistical analysis

All obtained data were subjected to three-way analysis of variance in CoStat software (CoStat ver. 6.45; CoHort Software) to examine the impact of drought stress (well-irrigated and drought stress), SA application (SA treated and SA-untreated), K fertilization (K_1, K_2 and K_3) and their interactions on response variables. Significant differences between means were assessed using Duncan Multiple Range Test at the 0.05 probability level.

4.3 Results

4.3.1 Grain yield and yield components

Drought stress significantly decreased the number of fertile tillers, 1000 kernel weight, and grain yield per plant (Table 4.1). SA application increased the number of fertile tillers under well-irrigated and drought stress conditions. Combined SA and K_3 treatments resulted in the production of a higher number of fertile tillers under well-irrigated condition, while K_1 treatment in SA-untreated plants recorded the lowest number of fertile tillers under drought stress condition. K_3 treatment recorded the higher number of fertile tillers under drought stress condition, while combined SA and K_3 treatments (SA+K_3) resulted in a greater increase in the number of fertile tillers. Application of SA+K_3 increased number of fertile tillers by 18.5 % compared to SA-untreted+K_1 treatments under drought stress condition. The 1000 kernel weight was higher under well-irrigated condition irrespective of SA and K treatments. SA application slightly enhanced 1000 kernel weight under drought stress condition compared to SA-untreated plants. Different levels of K did not affect 1000 kernel weight under both well-irrigated and drought stress conditions. SA+K_3 treatments recorded a higher value of 1000 kernel weight under both well-irrigated and drought stress conditions.

Application of SA+K_3 increased the 1000 kernel weight by 9.26 % compared to SA-untreted+K_1 under drought stress condition.

Grain yield was significantly higher in SA treated plants compared to SA-untreated. The different levels of K did not affect grain yield significantly in SA-untreated plants under well-irrigated condition. Under drought stress condition, the K_1 treatment in SA-untreated plants recorded the lowest grain yield, while the K_3 treatment improved grain yield. SA+K_3 treatments significantly increased grain yield by 14.4 and 13.3 compared to SA-untreated and K_1 treatment, under both well-irrigated and drought stress conditions, respectively.

4.3.2 Grain mineral content

Grain mineral content was significantly influenced by SA and K application under drought stress condition (Table 4.2). The total K content in grains was higher in SA-treated plants under drought stress condition, while the lowest K content was observed under well-irrigated condition irrespective of SA and K treatments. The K_3 treatment did not enhance K content in SA untreated plants under well-irrigated condition, but SA application with K_3 and K_2 treatments significantly increased K content under drought stress condition. Combined SA and K_3 treatments resulted in the highest K content under drought stress condition. With respect to N, drought stress significantly enhanced grain N content. Higher total N content was observed in SA-treated plants under drought stress condition. Although various levels of K did not significantly affect total N content under well-irrigated condition, the combined SA and K_3 treatments resulted in a higher value of total N content under drought stress condition compared to SA-untreated. Drought stress significantly increased grain P content. Different levels of K fertilization did not significantly affect grain P content, however SA-treated plants recorded slightly higher grain P content under well-irrigated as well as drought stress conditions. Drought stress increased grain inorganic phosphorus (Pi) content. Different levels of K fertilization did not significantly influence grain Pi content. While, SA application slightly increased grain Pi content particularly when it combined with K_3 treatment. Grain Mg content was higher under drought stress condition irrespective of SA and K treatments. The combined SA and K_3 treatments recorded higher value of Mg content, however the difference was statistically insignificant. Grain Ca content increased under drought stress condition. K_2 and K_3 treatments remarkably increased grain Ca content, and the highest Ca content was obtained under SA+K_3 and SA+K_2 treatments. Zn content was higher under drought stress condition irrespective of SA and K treatments. In general, SA+K_3 treatments increased grain K, N, P, Pi, Mg, Ca and Zn contents of grains by 11.1, 9.6, 8.9, 15.3, 11.1, 42.3 and 4.0%, respectively, compared to SA-untrated+K_1 treatments under drought stress condition.

4.3.3 Grain starch and crude protein content

Drought stress reduced the starch content under all levels of K treatments, and the reduction was greater in SA-untreated plants (Table 4.3). Application of SA enhanced grain starch content under drought stress condition. Different levels of K fertilization did not affect grain starch content significantly. The lowest starch content was observed in SA-untreated plants of K_1 treatment, while combined SA and K_3 treatments resulted in a higher starch content. SA+K_3 treatments increased grain starch content by 12.2% under drought stress condition, compared to SA-untreated+K_1 treatments.

Crude protein content was recorded higher under drought stress condition. Application of SA did not affect grain crude protein content under well-irrigated condition, however it significantly increased crude protein content under drought stress condition.

Different levels of K fertilization did not significantly affect grain crude protein content under well-irrigated as well as drought stress condition. Application of SA in combination with K_3 treatment significantly increased grain crude protein under drought stress condition. The highest level of crude protein was observed under drought stress condition in SA treated plants with K_3 treatment. SA+K_3 treatments increased grain crude protein content by 9.7% compared to SA-untreated+K_1 treatments under drought stress condition.

4.3.4 Grain TP and WSP content

Total pentosan content was significantly increased under drought stress compared to well-irrigated condition irrespective of SA treatment and various levels of K fertilization. SA application alone did not affect the total pentosan content under drought stress condition (Figure 4.1), while combined SA and K_3 treatments slightly increased grain total pentosan content. SA+K_3 treatments increased grain total pentosan content by 7.55% compared to SA-untreated+K_1 treatments under drought stress condition. Water-soluble pentosan content was slightly reduced under drought stress condition in SA-untreated plants where a low K fertilizer was also applied. Application of SA alone did not affect grain water-soluble pentosan content, while K_3 treatment slightly enhanced water-soluble pentosan content under both well-irrigated and drought stress conditions. A higher value of water-soluble pentosan was observed with combined SA and K_3 treatments. SA+K_3 treatments increased grain water-soluble pentosan by 20.3% compared to SA-untreated+K_1 treatments under drought stress condition.

4.3.5 Grain Phy- P content

Drought stress increased grain Phy-P content by 13.4% compared to well-irrigated condition (Table 4.3). K_3 treatment recoded the highest Phy-P content under drought stress condition.

SA application did not affect grain Phy-P content, significantly. However, combined SA and K treatments influenced the percentage of Phy-P to total P. The percentage of Phy-P to total P content was remarkably decreased by SA+K_3 treatments compared to SA-untreated under drought stress condition.

4.4 Discussion

Application of SA either through seed soaking, adding to the nutrient solution, irrigating, or spraying was found to improve abiotic stress tolerance mechanisms in plants under environmental stress conditions (Gondor *et al*. 2016, Horvath *et al*. 2007; Khan *et al*. 2015; War *et al*. 2011). Foliar application as spraying SA on leaves might be an appropriate method to attenuate the adverse effects of post-anthesis drought stress. Potassium (K) is an influential macro-nutrient which plays a key role in improving plant water status, stomatal movements, enzyme activity, osmoregulation and membrane stability that may help the plants to tolerate the adverse effect of drought stress (Ahmad *et al*. 2014; Jatav *et al*. 2014; Erel *et al*. 2015). In the present study the combined effect of SA foliar application and various levels of K fertilization on wheat productivity and grain quality was evaluated. The results of this study indicated that drought stress at anthesis and grain filling stages considerably influenced crop growth, and yield, by affecting all yield components (number of fertile tillers per plant, and 1000 kernel weight) which significantly decreased the grain yield. Decrease in grain yield under drought stress condition may be due to the reduction in the duration and rate of grain filling processes which resulted in the production of small grains and consequently reducing grain yield. Machado *et al*. (1993) indicated that drought stress at near anthesis and grain filling stage decreased translocation of photosynthetic products and resulted in wrinkled grains which reduced 1000 kernel weight and decreased grain yield. In this study combined application of adequate amount of K and SA enhanced grain yield and yield components under drought stress condition. K plays a vital role in plant growth and productivity by regulating photosynthesis, stomatal opening, transportation of assimilates from source to sink, enzyme activity and carbohydrate synthesis (Daniel *et al*. 2016; Yawson *et al*. 2011; Lakudzala 2013). SA is also essential for plant growth, physiological performance and crop productivity under abiotic stress conditions. It can regulate transpiration rates, stomatal regulation and photosynthetic processes, and ion uptake and transport in plants (Gunes *et al*. 2005; War *et al*. 2011). Many studies indicated that grain yield and yield components were increased when K or SA was applied separately (Aown 2012; Sharafizad *et al*. 2013; El Sayed *et al*. 2016; Yavas and Unay 2016;), however for the first time, it is observed in this study that combined application of SA and K improved plant growth and grain yield of wheat greater than when SA and K were applied separately.

Grain mineral content was affected by drought stress significantly. In present study K, N, P, Pi, Ca Mg, and Zn contents increased under drought stress condition. Except for Zn, this increase was greater in SA-treated plants where a high dose of K fertilizer (K_3 treatment) was also applied. SA and K levels had no effect on Zn content under drought stress condition. Combined application of SA and K_3 treatment significantly increased grain K, N and Ca contents. Plant growth was reduced in drought stressed plants, and thus minerals might have been transported from older leaves to the meristematic tissues and developing grains. Raza *et al.* (2013) found that application of K improved the uptake of K, N, P and Ca in wheat under drought stress condition. Application of SA increased minerals contents in wheat under drought stress condition, and combined application of SA and a high dose of K fertilizer (K_3 treatment) significantly increased grain K, N and Ca contents. This increase in K, N and Ca contents in wheat might be linked with drought mitigating effects of SA treatment. SA regulates ion uptake in plants, and it was observed that application of SA increased K, N, P and Mg content in maize under drought condition (Gunes *et al*. 2005). Alam (1994) revealed that drought stress increased N content in plants, and this increase was due to the accumulation of free amino acids that are not synthesized into protein. Patel and Singh (1998) also observed that under drought stress condition, most of the N and P was accumulated in grains. A higher of grain minerals contents particularly N, K, Ca and Pi may improve the nutritional quality of bakery products specially, the bread which is consumed largely in developing countries. In present study, it was found that grain Pi content slightly increased under drought stress condition, and SA application resulted in a higher value of grain Pi content. There has been insufficient research work on effect of drought, and combined treatment of SA and K on grain Pi content but overall, a positive relation was observed between total P and Pi content in this study, which could be a reason for a relatively higher grain Pi content in SA applied plants.

Starch is the most abundant component present in the grain endosperm of wheat (Lineback and Rasper, 1988). Grain starch was remarkably reduced under drought stress condition. This reduction may have been due to a reduction in plant photosynthetic activity, and therefore carbohydrate synthesis. Saeedipour (2011) reported that drought stress during anthesis and grain filling significantly reduced starch content of wheat. An adequate level of K fertilizer alleviates the adverse effects of drought and increases grain starch under both well-irrigated and drought stress condition (Zareian *et al*. 2014; Fayez and Bazaid 2014). Application of SA in combination with K_3, greatly enhanced grain starch content of wheat under well-irrigated and drought stress conditions. This increase in starch content, might be due to the combined effect of SA and K which regulated stomatal closure, improved photosynthetic activity, enhanced carbohydrate transportation from leaves to grains, and eventually resulted in a high grain starch content.

Grain crude protein was increased under drought stress condition. Irrigation may decrease flour protein content by dilution of N with carbohydrates (Guttieri *et al*. 2005). Similarly, Singh *et al*. (2012) reported a significant increase in protein content of wheat grains grown under rainfed condition. An elevated level of K slightly increased crude protein under drought stress condition. Application of K improves plant N uptake and utilization, enzyme activity, and protein synthesis (Daniel *et al*. 2016; Lakudzala 2013). In present study, application of SA and K_3 enhanced crude protein content in wheat under drought stress condition. Kumar *et al*. (1999) reported that SA treatment increased total protein in soybean grains, and this increase might be because of enhanced activity of nitrate reductase by SA application. Increase in grain protein content has a positive effect on nutritional and bread-making quality of wheat flour.

Drought stress significantly increased grain total pentosan irrespective of SA acid and K treatments. K_3 treatment slightly enhanced total pentosan content under drought stress condition. SA partially induced total pentosan content under well-irrigated condition. Effect of K fertilization and SA treatment on grain pentosans under drought stress condition, was not reported by earlier researchers. It is assumed that under drought stress, plants produced less number of fertile tillers, and most of this non-starch polysaccharides might be accumulated in grains. Chunxi *et al*. (2002) indicated that ecological environment was a crucial factor for pentosan content in wheat. Rakszegi *et al*. (2014) found that the content of arabinoxylan was generally increased by drought stress in drought sensitive varieties of wheat. The present study indicated that water-soluble pentosan content was slightly lower under drought stress condition, particularly with K_1 treated plants. Water-soluble pentosan increases the viscosity and the stability of dough foam structure. It helps in bigger loaf volume and a finer homogeneous bread crumb (Courtin and Delcour 2002). It was observed in present study that combined application of SA and a high dose of K fertilizer (K_3 treatment) to some extent was enhanced carbohydrate formation, and consequently, increased grain water-soluble pentosan content under drought stress condition.

Phy-P (*myo*-inositol 1,2,3,4,5,6-hexakisphosphate) serves as seed storage for phosphorus, but this compound can contribute to the nutritional deficiencies when seeds are used as food (Rosa 1999; Raboy 2001). It binds with proteins and important minerals such as Fe, Ca and Zn, and reduces their availability (Raboy 2001) Phy-P content was increased with higher K fertilization and drought stress condition. Application of SA did not affect grain Phy-P content under drought stress condition, but interestingly, the percentage of Phy-P to total P content was reduced by SA application. The reason for the reduction in Phy-P to total P content is unclear, however, it is assumed that SA application under drought stress condition enhances plant growth, and total P might have been accumulated in growing prats of plant or stored as ATP to induce drought tolerance and improve the seed vigor to regrow in the next season. Miura and Tada (2014) reviewed that proteins involved in the synthesis of ATP are up-regulated by SA and drought, due to an increase in growth and to withstand drought stress. In present study, the decrease in the percentage of Phy-P to total P content may explain the increase of Pi content under combined SA and K_3 treatments. Thus, from present study, it was found that combined K and SA application may decrease the share of total P in Phy-P and increases bioavailability of Pi that may improve the grain nutritional quality.

4.5 Summary

In this chapter, the effects of combined application of SA and different levels of K fertilizer on growth, grain yield, grain mineral content and nutritional quality of wheat under drought stress condition was evaluated. The result showed that, drought stress decreased grain yield by 41.1%, starch content by 10.2% and WSP content by 3.5% in comparison to well-irrigated condition. However, grain crude protein, TP and Phy-P content were increased by 33.0, 17.9, and 13.4% respectively. Under the same drought condition, the application of combined SA and high K levels has increased grain yield (9.7%), starch (12.2%) and WSP content (20.3%) compared to SA-untreated with low level of K fertilizer. In addition, SA application decreased the percentage of Phy-P to total P under drought stress. These results suggested that combined treatment of SA foliar application and a higher doses of K fertilizer can partially improve wheat productivity, grain nutritional quality, particularly WSP that influences the bread-making quality, without increasing the anti-nutrient component phytate under drought stress condition.

Table 4.1 Effect of combined application of SA and K on yield components, and grain yield of wheat under drought stress condition. Means with different letters are significantly different from each other at $p < 0.05$.

Stress levels	**SA treatment**	**K Levels**	**Number of fertile tillers (plant^{-1})**	**1000 kernel weight (g)**	**Grain yield per plant (g)**
Well-irrigated	+SA	K_1	25.00^{abc}	41.72^{a}	55.72^{b}
		K_2	27.00^{ab}	41.90^{a}	57.01^{ab}
		K_3	28.25^{a}	42.71^{a}	59.49^{a}
	-SA	K_1	24.00^{c}	41.65^{a}	51.99^{c}
		K_2	24.75^{bc}	41.86^{a}	52.66^{c}
		K_3	26.00^{abc}	42.43^{a}	54.51^{c}
Drought stress	+SA	K_1	17.75^{de}	38.55^{b}	32.65^{e}
		K_2	18.75^{de}	38.79^{b}	33.35^{de}
		K_3	19.25^{d}	39.41^{b}	34.19^{d}
	-SA	K_1	16.25^{e}	36.07^{c}	30.17^{f}
		K_2	17.00^{de}	36.24^{c}	31.36^{e}
		K_3	17.25^{de}	37.71^{c}	31.98^{e}

Table 4.2 Effect of combined application of potassium and SA on grain mineral content of wheat under drought stress condition. Means with different letters are significantly different from each other at $p < 0.05$.

Stress levels	**SA treatment**	**K Levels**	**K (mg g^{-1})**	**N (mg g^{-1})**	**P (mg g^{-1})**	**Pi (mg g^{-1})**	**Mg (mg g^{-1})**	**Ca (µg g^{-1})**	**Zn (µg g^{-1})**
Well-irrigated	+SA	K_1	7.07^{d}	26.77^{c}	4.02^{d}	0.255^{de}	0.957^{cde}	186.79^{de}	54.97^{bc}
		K_2	7.09^{d}	27.11^{c}	4.14^{cd}	0.269^{cde}	0.965^{bcde}	194.81^{de}	56.83^{bc}
		K_3	7.75^{c}	27.42^{c}	4.38^{bcd}	0.288^{abcde}	0.995^{bcde}	215.98^{d}	58.03^{b}
	-SA	K_1	6.70^{d}	26.71^{c}	3.84^{d}	0.238^{e}	0.896^{e}	110.13^{j}	49.90^{c}
		K_2	6.81^{d}	27.13^{c}	3.87^{d}	0.254^{de}	0.924^{de}	131.40^{fg}	52.83^{bc}
		K_3	7.00^{d}	27.23^{c}	4.04^{d}	0.284^{bcde}	0.949^{cde}	162.41^{ef}	53.89^{bc}
Drought stress	+SA	K_1	8.38^{ab}	35.76^{ab}	4.66^{abc}	0.333^{abc}	1.057^{abcd}	307.94^{b}	71.01^{a}
		K_2	8.50^{a}	36.12^{ab}	4.76^{ab}	0.346^{ab}	1.130^{ab}	357.35^{a}	72.94^{a}
		K_3	8.61^{a}	37.63^{a}	5.01^{a}	0.355^{a}	1.161^{a}	367.39^{a}	75.26^{a}
	-SA	K_1	7.75^{c}	34.35^{b}	4.60^{abc}	0.308^{abcd}	1.045^{abcd}	258.27^{c}	71.72^{a}
		K_2	7.87^{bc}	34.37^{b}	4.69^{ab}	0.329^{abc}	1.078^{abcd}	303.29^{b}	72.67^{a}
		K_3	7.91^{bc}	35.52^{b}	4.79^{ab}	0.338^{ab}	1.099^{abc}	312.32^{b}	73.19^{a}

Table 4.3 Effect of combined application of potassium and SA on grain starch, crude protein and Phy-P content of wheat under drought stress condition. Means with different letters are significantly different from each other at $p < 0.05$.

Stress levels	**SA treatment**	**K Levels**	**Starch (%)**	**Crude protein (%)**	**Phytate P ($mg\ g^{-1}$)**
Well-irrigated	+SA	K_1	67.30^{abcd}	14.67^{c}	2.87^{c} (71.4)
		K_2	69.19^{ab}	14.82^{c}	2.99^{c} (72.2)
		K_3	72.31^{a}	15.00^{c}	3.05^{bc} (69.6)
	-SA	K_1	66.12^{abcde}	14.60^{c}	2.86^{c} (74.5)
		K_2	67.72^{abc}	14.84^{c}	2.98^{c} (77.0)
		K_3	69.28^{ab}	14.89^{c}	3.03^{bc} (75.0)
Drought stress	+SA	K_1	61.53^{cdef}	19.56^{ab}	3.28^{abc} (70.4)
		K_2	64.40^{bcdef}	19.75^{ab}	3.36^{ab} (70.6)
		K_3	65.39^{abc}	20.58^{a}	3.53^{a} (70.5)
	-SA	K_1	58.28^{c}	18.76^{b}	3.26^{ab} (70.9)
		K_2	60.02^{ef}	18.86^{b}	3.34^{ab} (71.2)
		K_3	60.37^{def}	19.43^{ab}	3.51^{a} (73.3)

* Values in parentheses are percentage of phytate P to Total P content

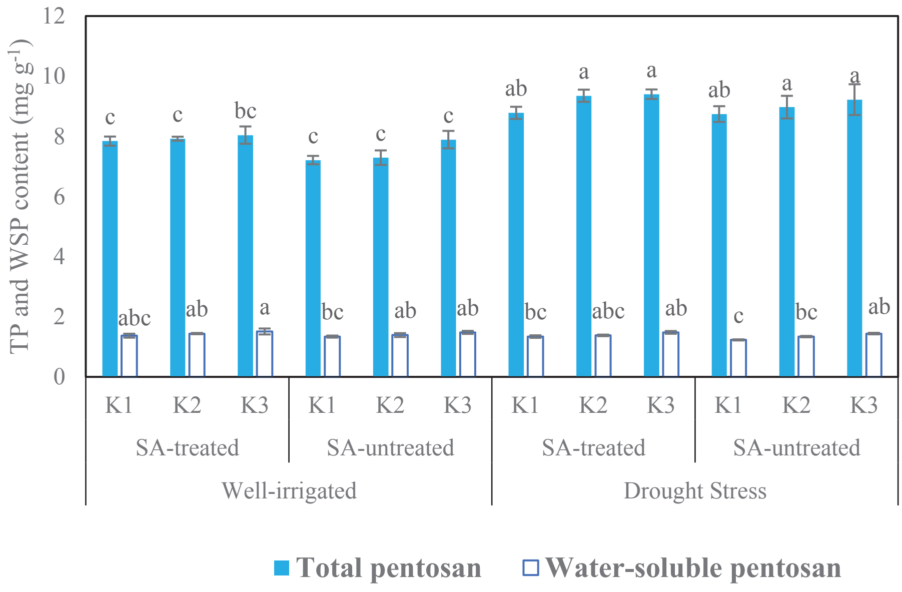

Fig. 4. 1 Effect of combined application of SA and K on grain total pentosans (TP) and water soluble pentosan (WSP) of wheat under drought stress condition. The same letter indicates no significant difference ($p < 0.05$).

Table 4.4 Significance of different source of variance.

Parameters	Source of variation						
	DS	SA	K	DS x SA	DS x K	SA x K	DS x SA x K
No. of fertile tillers (plant^{-1})	<0.001	<0.001	0.004	0.926	0.449	0.688	0.892
1000 kernel weight (g)	<0.001	0.009	0.109	0.019	0.925	0.948	0.849
Grain yield (g plant^{-1})	<0.001	<0.001	<0.001	0.032	0.044	0.041	0.050
Grain N content (mg g^{-1} DW)	0.092	<0.001	0.258	0.123	0.949	0.654	0.973
Grain K content (mg g^{-1} DW)	<0.001	<0.001	0.004	0.926	0.449	0.688	0.892
Grain P content (mg g^{-1} DW)	0.063	<0.001	0.081	0.458	0.799	0.992	0.985
Grain Pi content (mg g^{-1} DW)	0.202	<0.001	0.108	0.839	0.945	0.833	0.976
Grain Mg content (mg g^{-1} DW)	0.141	<0.001	0.153	0.596	0.933	0.943	0.566
Grain Ca content (mg g^{-1} DW)	<0.001	<0.001	<0.001	0.443	0.891	0.220	0.737
Grain Zn content (mg g^{-1} DW)	0.073	<0.001	0.162	0.159	0.946	0.957	0.853
Grain Starch content (% DW)	0.020	<0.001	0.086	0.358	0.844	0.859	0.987
Grain crude protein content (% DW)	0.092	<0.001	0.257	0.123	0.949	0.655	0.973
Grain TP content (mg g^{-1} DW)	0.0370	0.005	0.042	0.381	0.662	0.686	0.714
Grain WSP content (mg g^{-1} DW)	0.102	<0.001	0.060	0.527	0.780	0.763	0.788
Grain Phy-P content (mg g^{-1} DW)	0.309	0.087	0.0261	0.520	0.784	0.438	0.485

Probability values in the table are related with three-way analysis of variance for factors drought stress (DS), salicylic acid (SA), potassium (K), and the interaction of drought stress x salicylic acid (DS x SA), drought stress x potassium (DS x K), salicylic acid x potassium (SA x K) and drought stress x salicylic acid x potassium (DS x SA x K). (Significance at $p < 0.05$)

Chapter 5

General discussion

The changes in the climate under the influence of global warming are creating unusual weather phenomena often all over the world in the form of drought. Drought stress decreases plant water potential and turgor to the extent that plants face difficulties in performing normal physiological functions, and as a result, cell enlargement decreases leading to growth inhibition and reproductive failure (Seyed *et al*. 2012). Drought can affect plants at any growth stage, however grain filling is one of the most sensitive growth stages of wheat to drought stress (Bradford 1994). Drought during grain filling can limit the rate and duration of seed set and grain filling processes, causing smaller grain size, earlier physiological maturity, reduced number of grains, lower grain weight and lower yield of wheat (Gupta *et al*. 2001).

5.1.1 Effect of NPK fertilization on grain yield, nutritional and anti-nutritional quality, and seed germination of wheat

It is estimated that agricultural production should be increased by 70 percent, to feed the world population which is expected to surpass 9 billion in the year 2050 (WSFS 2009). Enriching soil fertility by mineral fertilization is one of the main approaches to increase crop production especially cereals, to meet the demand and ensure food security for rapidly growing population all over the world. Application of an appropriate rate of NPK fertilization improves plant productivity and affects grain nutritional quality. Investigation into the effects of macronutrients especially NPK on the grain yield, quality, anti-nutrient compounds, and seed quality attributes, deserves more attention. Therefore, the study in chapter 2 was carried out to find the effect of combined NPK fertilization on grain yield, grain mineral, grain nutritional quality, grain gluten, TP, WSP and Phy-P content of wheat. Also, the effect of mother plant NPK nutrition on seed germination and physiological activity was also evaluated. The results indicated that a higher level of NPK fertilization significantly improved plant growth. It also increased grain yield, mineral content, crude protein content, gluten content, WSP, and Phy-P content of wheat. The grain yield of wheat and other cereal crops increased with the application of a high rate of N, P, and K fertilizers

(Hussain *et al*. 2002; Laghari *et al.* 2010; Abdel-Aziz *et al*. 2016). A higher level of NPK fertilization enhanced grain mineral content (Laghari *et al*. 2010; Campillo *et al*. 2010). Crude protein content was increased with increase in NPK rate (Sameen *et al*. 2002). It was observed that, TP content decreased with increase in NPK rate, whereas WSP content was significantly increased. Jing *et al*. (2010) found that N fertilization increased pentosan content of waxy wheat under water logging condition. A higher rate of NPK fertilization can significantly increase the level of gluten content in wheat grain (Tanacs *et al*. 2005). Increased NPK rate resulted in a higher grain Phy-P content and this increase may be due to a higher level of P fertilization. Application of P fertilizer increased grain total P content in wheat (Ali *et al*. 2014) and it may contribute to the synthesis and accumulation of higher phytate content.

5.1.2 Effect of mother plant NPK nutrition on seed germination and physiological performance of the second generation

The seeds obtained from the first experiment were evaluated for germination percentage, and physiological activity. It was observed that mother plant NPK nutrition improved seed germination, seedling growth, and physiological performance. Seeds from the plants that obtained a higher level of fertilizer and irrigation during the production stage exhibited greater seedling establishment compared to the seeds from the plants that obtained a lower level of fertilizer and irrigation (Hampton 1992). Application of a higher rate of NPK improved seed quality and resulted in a higher seed germination percentage in China aster (Doddagoudar *et al*. 2004). Phytase activity reached a maximum level on the 6th day of germination and as a result T_3 and T_2 recorded higher values of phytase compared to T_1. The high phytase level of T_3 and T_2 during the germination period might be due to a high P and protein content in the mother plant grains compared to the control (T_1). The increase in phytase level may be due to de novo synthesis of the enzyme during germination (Sung *et al*. 2005). Phytase level started to decrease slightly on the 7th day of germination. Houde *et al*. (1990) concluded that, the decrease in phytase activity, might be due to the degradation of this enzyme by active protease. A negative relationship between phytase activity and Phy-P content was observed during seed germination; as phytase activity increased the Phy-P content decreased. During seed germination, phytate is degraded by the phytase enzyme in cereals (Kumar *et al*. 2010). The highest Pi content was observed on 7th day of germination. Phytase in germinating seeds removes orthophosphate groups from the inositol ring of phytate to produce free Pi, and a chain of intermediate myo-inositol phosphates (Debnath *et al*. 2005), that may enhance P availability.

5.2 Effect of drought stress and NPK fertilization on grain yield, nutritional and anti-nutritional quality of wheat

Limitations rising from drought stress during the upcoming decades due to climatic change may reduce precipitation and accelerate evapotranspiration (World Bank 2007; Lobell *et al*. 2008). Drought stress negatively affects plant growth, decreases grain yield, reduces food availability, and therefore, leading to food scarcity and insecurity. As explained in chapter 2, the results of this investigation showed that, application of NPK improved wheat productivity and grain nutritional quality under normal condition. Therefore, the experiment in chapter 3 was conducted to evaluate the effects of combined application of NPK on wheat growth, productivity, grain nutritional quality, TP, WSP, and Phy-P content under drought stress condition. The result of this study indicated that, drought stress significantly decreased plant growth and grain yield. Drought stress at flowering stage decreases grain-set due to lower fertilization which is caused by pollen sterility and ovule abortion (Hossain *et al*. 2012). An increased rate of NPK fertilization mitigated the drought stress detrimental effects and resulted in production of higher grain yield under drought stress condition. Ati *et al*. (2016) also found that application of NPK fertilizer increased number of spikes and grain yield of wheat under drought stress condition. Application of a high rate of N fertilizer improves water use efficiency and enables the plants to survive and thus produce high grain yield under drought stress condition (Akram *et al*. 2014).

In this study grain total N, K, P, Pi, Mg and Ca content were increased under drought stress condition. The reason for increased grain mineral content may attribute to decrease in plant growth which resulted in mineral transportation from older leaves to the meristematic tissues and developing grains. A higher rate of NPK fertilization partially increased grain mineral content except for Zn. The F_3 treatment which consisted of a higher rate of N, P and K fertilizers enhanced N, P, K content in wheat grains. Application of a high dose of K, improves K, N, P and Ca uptake of wheat under drought stress condition (Raza *et al*. 2013). Grain Pi content was slightly increased with increase in NPK rate. There was a positive relation between total P and Pi content in this study, which could be a reason for a high Pi content. The P uptake of wheat is enhanced when the dose of P fertilizer increases (Saha *et al*. 2014). Minaminokaori cultivar recorded a higher grain Mg content under well-irrigated and mild stress conditions. A higher NPK fertilization enhanced Mg content under well-irrigated condition. N fertilization exhibits more expressive effect on plant Mg uptake under optimal water condition (Slamka *et al*. 2011). Grain Zn content was higher in Minaminokaori compared to Lalmi-2 irrespective of the level of drought stress and NPK treatments. The reason for this may be higher grain yield in Lalmi-2 compared to Minaminokaori and thus grain Zn content was decreased in Lalmi-2.

Grain starch content was significantly decreased in both cultivars under drought stress condition. Plant photosynthetic activity is decreased under drought stress, and therefore carbohydrate formation and accumulation of starch is declined. Saeedipour (2011) observed that drought stress during anthesis and grain filling decreases starch content of wheat. Application of NPK did not enhance grain starch content under both well-irrigated and drought stress conditions. Kindred *et al.* (2008) found that N fertilization decreased the starch content of wheat grain. A higher grain starch content was recorded in control plants where fertilizer was not applied (Hlisnikovsky and Kunzova 2014). It was observed that crude protein content increased under drought stress condition in both cultivars. Under well-irrigated condition, flour protein content may decrease due to dilution of N with carbohydrates (Guttieri *et al*. 2005). NPK fertilization increased the synthesis of the raw protein in wheat (Crista 2012). In this study, F_3 treatment consisted an elevated level of N and K fertilizers that resulted in a higher crude protein content. Adequate amount of K fertilization improves plant N uptake and protein synthesis (Lakudzala 2013; Daniel *et al*. 2016). Minaminokaori recorded a higher grain crude protein content compared to Lalmi-2 which may be due to high yield of Lalmi-2 cultivar and varietal difference in uptake of N and protein synthesis.

Mild drought stress condition increased grain TP content to some extent, however a higher level of NPK fertilization slightly decreased TP content in the two cultivars under both well-irrigated and drought stress conditions. Plant produced less grain yield under drought stress, and therefore most of this non-starch polysaccharides might be accumulated in grains. The content of arabinoxylan generally increases under drought stress (Rakszegi *et al*. 2014). The WSP content was reduced under drought stress condition and NPK fertilization significantly partially increased WSP content under both well-irrigated and drought stress conditions.

Grain Phy-P content was increased with the severity of drought and increased rate of NPK fertilization. Phy-P is the major storage form of P in cereals, therefore the content of this antinutrient compound mostly depends on grain total P. The F_3 treatment which consisted of a higher rate of P fertilizer may have contributed to a higher Phy-p synthesis and accumulation in wheat grains. Raboy and Dickinson (1984) also concluded that application of P fertilizer might be one of the reasons for high phytate content.

5.3 Effect of combined salicylic acid and potassium application on grain yield, nutritional and anti-nutritional quality of wheat under drought stress condition

Production of field crops decrease due to detrimental effects of climate change, global warming and drought stress. Under such circumstances, it is necessary to minimize the losses and ensure food security for the rapidly growing population. Drought stress severely impairs plant growth and development, reduces plant production and limits performance of crop plants, more than any other environmental factor (Shao *et al*. 2009). Drought stress causes impaired mitosis; cell elongation and expansion which results in reduced growth and yield (Hussain *et al*. 2008). It constrains crop production and quality seriously by affecting the growth, dry mater and harvestable yield in plants (Anjum *et. al*. 2011).

Salicylic acid (SA) is involved in the regulation of many plant physiological processes such as photosynthesis, proline and nitrogen metabolism, glycine betaine synthesis, antioxidant defense system, and plant water relations under stress condition and thereby protects plants against abiotic stresses (Khan *et al.* 2013, 2014; Nazar *et al*. 2011; Miura and Tada 2014). Exogenous application of SA may enhance plant performance under drought stress by modulating various physiological and biochemical processes which are negatively affected by drought stress (Pirasteh *et al*. 2012). SA affects the plant growth under stress through enhanced nutrient uptake, water relations, stomatal regulation and photosynthesis (Hayat *et al*. 2009), and therefore reduces yield loss under drought stress condition.

K is one of the essential elements and is required by the plant in large quantities for maintaining the osmotic balance and opening and closing of stomata. Enzymes like pyruvate kinase uses K as a cofactor. Application of K fertilizer improves water relations and crop productivity under drought stress condition (Islam *et al*. 2004). K fertilization alleviates detrimental effects of drought stress by improving many physiological processes such as regulation of turgor pressure and photosynthesis, enzyme activation and translocation of cations (Mengel and Kirkby 2001). K fertilization mitigates the injury of active oxygen derived from drought stress to plasma membrane and maintain the integrality of cell membrane, protecting enzyme activity in cells (Raza *et al*. 2014). Hence the drought tolerance of the plants could be enhanced through K fertilization. The individual effect of SA and K on yield, and physiological changes in wheat under drought stress condition was investigated by other researchers, however no studies were carried out on the effect of combined application of SA and K on productivity and nutritional quality of wheat under environmental stress such as drought. Therefore, the study in chapter 4 was conducted to ascertain the effect of combined application of SA and K fertilizer on wheat productivity and content of grain mineral, starch, crude protein, pentosans and Phy-P under drought stress condition.

The results of this study indicated that, drought stress at anthesis and grain filling stages significantly decreased wheat growth and grain yield. Cell elongation of higher plants can be inhibited under drought stress, by interruption of water flow from the xylem to the surrounding elongating cells (Nonami 1998). Drought stress at near anthesis and grain filling stage decreases translocation of photosynthetic products, which results in wrinkled grains, reduced 1000 kernel weight and decreased grain yield (Machado *et al*. 1993). Combined application of an optimal amount of K and SA enhanced grain yield and yield components under drought stress condition. SA is essential for plant growth, physiological performance and crop productivity under abiotic stress conditions. It can regulate transpiration rate, stomatal conductance, photosynthetic processes and ion uptake and transportation in plants (Gunes *et al*. 2005; War *et al.* 2011). K also plays a vital role in plant growth and productivity by regulating photosynthesis, stomatal opening, transportation of assimilates from source to sink, enzyme activity and carbohydrate synthesis (Daniel *et al*. 2016; Yawson *et al*. 2011; Lakudzala 2013), and thus it strengthens the drought tolerance and improves plant productivity under drought stress condition. It was observed that combined application of SA and K improved plant growth and grain yield of wheat better than SA and K were applied separately.

In this study K, N, P, Pi, Ca Mg, and Zn content increased under drought stress condition. Plant growth was impaired under drought stress, and consequently mineral were transported from older leaves to the meristematic tissues and developing grains which resulted in increased mineral content in grains. The increase was greater in SA-treated plants (except for Zn) where a high dose of K fertilizer was also applied. Application of K Improved K, N, P and Ca uptake of wheat under drought-stress condition (Raza *et al.* 2013). Combined application of SA and a high dose of K significantly increased K, N, P, Pi, Ca, and Mg content. SA regulates ion uptake in plants, and it was observed that application of SA increased K, N, P and Mg content in maize under drought condition (Gunes *et al*. 2005). Grain Pi content was increased under drought stress condition, and SA application further greatly enhanced Pi content. A positive relation was observed between total P and Pi content in this study, which could be a reason for a high Pi content in wheat grains.

Grain starch content was significantly decreased with increase in severity level of drought stress. The reduction in grain starch content could be attributed to impaired plant photosynthetic activity, and therefore a reduction in carbohydrate synthesis. Drought stress during anthesis and grain filling stages greatly reduced starch content of wheat (Saeedipour 2011). A high rate of K fertilization significantly alleviated the adverse effects of drought and increased grain starch content under both well-irrigated and drought stress conditions (Zareian *et al*. 2014; Fayez and Bazaid 2014). In this study, it was found that application of SA in combination with K_3, greatly enhanced grain starch content of wheat under both well-irrigated and drought stress conditions. Such increase in grain starch content of wheat might be due to the combined effect of SA and K which regulated stomatal closure, improved photosynthetic activity and enhanced carbohydrate transportation from leaves to grains. SA affects, water relations, stomatal regulation and photosynthesis under stress condition (Hayat *et al*. 2009), and therefore may enhance grain starch content.

Drought stress condition boosted total N uptake and thus increased grain crude protein. Guttieri *et al*. (2005) found that irrigation may reduce flour protein content by dilution of N with carbohydrates. A higher rate of K fertilization increased crude protein under drought stress condition. K fertilizer improves plant N uptake and utilization, enzyme activity, and protein synthesis (Daniel *et al*. 2016; Alam *et al*. 2009; Lakudzala 2013). Application of SA and K_3 enhanced crude protein content in wheat under drought stress condition. Application of SA increased total protein in soybean grains, and this increase might be due to enhanced activity of nitrate reductase by the SA treatment (Kumar *et al*. 1999).

Grain TP content was increased under drought stress condition, irrespective of the SA acid and K treatments. K_3 and K_2 recorded the highest TP content under drought stress condition. SA induced TP content under well-irrigated condition. It was assumed that under drought stress, plants produced less number of fertile tillers, and most of this non-starch polysaccharides were accumulated in grains. Rakszegi *et al*. (2014) found that the content of arabinoxylan was generally increased by drought stress in wheat drought sensitive varieties. WSP content was reduced under drought stress condition. Courtin and Delcour (2002) stated that WSP had a positive impact on the bread-making quality of wheat and the water-unextractable pentosan had a negative effect on the bread-making quality. This study has shown that a combined application of SA and K_3 treatment enhanced carbohydrate formation, and consequently, increased grain WSP content under both well-irrigated and drought stress conditions.

Drought stress and a higher rate of K fertilizer significantly increased grain Phy-P content. SA did not affect grain Phy-P content under drought stress condition, however interestingly, the percentage of Phy-P to total P content was reduced by SA application. In this study, the decrease in percentage of Phy-P to total P content may explain the increase of Pi content under combined SA and K_3 treatments. The results suggest that application of K and SA in combination may improve the grain nutritional quality by reducing grain Phy-P content, enhancing bioavailability of Pi and other minerals in wheat under drought stress condition.

5.4 Conclusion

This research was carried out to study the influence of mineral fertilizers such as N, P and K on growth, yield and grain quality of wheat under normal and drought stress conditions to investigate how to improve productivity and grain nutritional quality of wheat under drought stress condition. Moreover, the effect of combined application of SA and K on improvement of wheat productivity and grain quality under drought stress condition was also evaluated. The results revealed that NPK fertilization under well irrigated condition improved growth, grain yield, grain mineral content, crude protein, gluten and WSP content. It was observed that mother plant NPK nutrition level enhanced seed germination, seedling growth and physiological performances compared to control (Chapter 2). A further increase in NPK dose significantly improved wheat productivity grain mineral content and nutritional quality under drought stress condition. Drought stress decreased grain yield and grain starch content, and negatively affected grain nutritional quality of wheat by enhancing grain Phy-P content (Chapter 3). Combined application of SA and a higher rate of K fertilizer also significantly mitigated the adverse effect of drought stress by improving growth, grain yield, grain mineral content, and grain nutritional quality in wheat (Chapter 4). Interestingly, application of SA did not increase Phy-P content of wheat under both well-irrigated and drought stress conditions.

Summary

Environmental stress significantly influences crop productivity, and therefore affects food security, availability and quality. Precipitation patterns are influenced by the climate change and seasons of drought reduce water availability and decreases plant production which consequently increases food prices. Rainfed wheat faces a problem of water shortages during grain filling period which results in low productivity and yield loss. Many attempts were made to overcome this problem through breeding programs and introducing drought tolerant wheat varieties, however yield loss due to drought stress is still unavoidable. Improvement of wheat productivity and nutritional quality under environmental stress conditions such as drought stress deserves more attention and elaborative research studies. There were no sufficient studies on effect of NPK fertilization on wheat productivity and grain quality under drought stress condition. Effect of combined application of SA and K on mitigating drought stress in wheat was also not reported so far. Therefore, this study was conducted to: (1) Assess the effect of NPK fertilization on agronomic performance, productivity, grain mineral content and content of starch, crude protein, TP, WSP, and Phy-P content in grains of wheat under irrigated (non-stressed) condition. (2) Evaluate the effect of mother plant NPK nutrition on seed germination, and physiological performance of wheat during germination period. (3) Determine the effect of NPK fertilization on growth, grain yield, and grain nutritional quality of wheat under drought stress condition. (4) Find out the effect of combined application of SA and different levels of K fertilization on productivity and nutritional quality of wheat under drought stress condition. (5) Determine how to improve drought tolerance and enhance wheat productivity and grain nutritional quality under drought stress condition.

1. Effect of NPK fertilization on grain yield, nutritional and anti-nutritional quality, and seed germination of wheat

To evaluate the effect of NPK fertilization on growth, grain yield, grain mineral content, grain nutritional quality and content of gluten, pentosans, and Phy-P in wheat under normal

irrigation (non-stressed) condition; Three levels of NPK fertilizers (T_1: control, T_2:110 kg N + 60 kg P_2O_5 + 55 kg K_2O ha^{-1}, and T_3: 200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}) were applied (in chapter 2 of this study). The results indicated that a higher level of NPK (T_3) fertilization increased grain yield, crude protein, WSP, and dry gluten content, up to 151.6, 65.3, 40.5, and 408.9% compared to the control, respectively. It also enhanced the grain mineral content, however did not affect significantly on the grain starch content. Grain Phy-P content was increased with a higher NPK fertilization level and interestingly the level of phytase enzyme was also increased up to 46% in T_3 compared to the control. In a laboratory experiment the effect of mother plant NPK nutrition on seed germination and physiological performance was also determined. The results revealed that, mother plant NPK fertilization enhanced seed germination percentage, seedling fresh weight, phytase activity, Pi content, and Phy-P metabolism during the germination period suggesting that higher grain yield, improved grain quality, higher seed germination, improved seedling establishment, and enhanced physiological performances of seedlings of wheat could be achieved with using an appropriate level of NPK fertilization.

2. **Effect of drought stress and NPK fertilization on grain yield, nutritional and anti-nutritional quality of wheat**

To ascertain the effect of combined application of nitrogen N, P and K fertilizers on productivity, and nutritional quality of wheat under drought stress condition, the experiment in chapter 3 was designed with two wheat cultivars (Minaminokaori and Lalmi-2), and 3 levels of NPK fertilization: F_1 (150 kg N + 100 kg P_2O_5 + 75 kg K_2O ha^{-1}), F_2 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}), and F_3 (250 kg N + 140 kg P_2O_5 + 125 kg K_2O ha^{-1}). These fertilizers were applied in pots, as F_1 (2 g urea+3.76 g SSP+0.96 g potassium sulfate pot^{-1}), F_2 (2.8 g urea+4.51 g SSP+1.28 g potassium sulfate pot^{-1}) and F_3 (3.5 g urea+5.3 g SSP+1.6 g potassium sulfate pot^{-1}). Both cultivars were grown in pots in a greenhouse and irrigated regularly until early grain filling stage. Then the plants were exposed to 2 levels of drought stress with a well-irrigated control (in experiment chapter 3). The results of this experiment indicated that drought stress significantly decreased grain yield, grain starch and WSP content, but increased grain crude protein, TP, and Phy-P content of both cultivars. Lalmi-2 showed a greater tolerance to drought condition exhibiting a higher grain yield, and higher total K and starch content under both well-irrigated and drought stress conditions than Minaminokaori. In contrast, Minaminokaori recorded a higher grain mineral content and higher content of crude protein, TP, WSP and Phy-P than Lalmi-2. It is concluded that increase in rate of NPK fertilization could ameliorate the adverse effects of drought stress and enhance plant productivity. At the same time, it helped to increase content of minerals, crude protein and WSP content in the grain under drought stress condition. Besides applying higher rates of NPK fertilizers, it is suggested that use of fertilizer responsive and drought-tolerant genotypes such as Lalmi-2 will be beneficial to minimize the risk of yield loss due to drought stress.

3. Effect of combined salicylic acid and potassium application on grain yield, nutritional and anti-nutritional quality of wheat under drought stress condition

The study in chapter 4 highlighted the combined effects of SA and K on yield and grain quality of wheat under drought stress condition. Wheat plants were grown in pots in a greenhouse and subjected to 3 levels of K fertilizer (50, 100 and 200 kg ha^{-1}). K was applied in pots as: K_1 (0.49 g pot^{-1}) K_2 (0.98 g pot^{-1}), and K_3 (1.96 g pot^{-1}). The plants were foliar sprayed with SA (0.7 mM) at heading stage, and then imposed to the drought stress until harvesting. Drought stress decreased grain yield by 41.1%, starch content by 10.2% and WSP content by 3.5% in comparison to control. However, grain crude protein content, TP content and Phy-P content were increased by 33.0, 17.9, and 13.4% respectively. Under the same drought condition, the application of combined SA and high K levels has increased grain yield (9.7%), starch content (12.2%) and WSP content (20.3%) compared to SA-untreated with low level of K fertilizer treatment. In addition, SA application decreased the percentage of Phy-P to total P under drought stress. These results suggested that combined treatment of SA foliar application and a higher doses of K fertilizer can partially improve wheat productivity, grain nutritional quality, particularly WSP that influences the bread-making quality, without increasing the anti-nutrient component phytate P under drought stress condition.

Conclusion

The results of this study indicate that higher rates of NPK fertilization under normal irrigation condition increase grain yield and increase content of grain mineral, crude protein, WSP, and dry gluten, however reduces TP content. NPK fertilization did not affect the level of starch content in wheat grain. NPK fertilization of the mother plant enhanced seed germination, seedling growth, and improved the physiological performance of germinating seeds compared to the control. Phytase activity, phytate degradation, and the release of Pi during seed germination was highly affected by mother plant NPK fertilization. Drought stress significantly reduced grain yield, negatively affected grain nutritional quality of wheat by increasing anti-nutrient compounds such as grain Phy-P and reduced starch content which is the main source of energy. A higher rate of NPK fertilization effectively attenuated the deleterious effects of drought stress by improving productivity and nutritional quality of wheat under drought stress condition. Application of SA and a higher level of K also significantly improved wheat performance, productivity, grain mineral content, grain starch content, crude protein content, and content of pentosans. Considering these observations, both approaches, either applying an adequate level of NPK fertilizers or combined SA and K application can be utilized for minimizing yield loss and improving grain quality in wheat under drought stress condition.

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