CHAPTER 5

General discussion

5.1 Rice yield and cropping season

Averaged across all genotypes, grain yield in the cool season was about 15% lower than in the summer season and 30% lower than in the rainy season (Figure 5.1). Rice in the cool season had the lowest filled grain weight and 1000 filled grain weight. As rice was grown under well irrigated conditions in all seasons and the varieties used were photoperiod insensitive, it is most likely that the differences in yield among seasons was due to temperature. The average minimum/maximum temperature at flowering to harvesting in this study were 22.4/32.3 ºC for the rainy season, 17.1/ 31.1 ºC for the cool season and 25.8 /37.1 ºC for the summer season. A number of studies have investigated the physiological mechanisms responsible for the low temperature depression of grain yield (Hayase *et al*., 1969; Satake, 1976; Satake *et al*., 1987, 1988). Low temperature induces spikelet sterility by impairing development leading to a shortage of pollen at anthesis, thus limiting pollination and consequently decreasing rice grainyield.

On the other hand, high temperature can also decrease rice grain yield by inducing sterility. Rice plants are most sensitive to excessive heat during two periods, at early flowering and the young microspore stage (Satake and Yoshida, 1978). Moreover, it has been reported that high temperatures (39 ºC) on the day of flowering decrease the ability of pollen grains to swell, thus resulting in poor anther dehiscence (Matsui *et al.*, 2000).

In a previous study there was a 7–8% yield decrease in rice for each 1 ºC increase in maximum/ minimum temperature from 28/21 to 34/27 °C (Baker *et al.*, 1992). Peng *et al*. (2004) noted that rice yield declined by 10% for each 1 ºC increase in minimum temperature. The results in Chapter 2, showed that grain yield and filled grain yield in the summer crop was depressed by ca. 10 to 20% compared to the rainy crop (Figure 5.1), supported these earlier studies. However, since the decrease in grain yield was smaller than those obtained in previous reports, it might be possible that *Indica* Thai rice varieties are more tolerant to high temperature in the summer but more sensitive to low temperature in the cool season.

5.2 Milling quality and cropping season

Milling quality was one of a number of quality traits investigated in this thesis and others are discussed separately in a later section. In Chapter 2, there was a higher percentage of grain chalkiness in the summer crop and clear differences among rice varieties. In particular, SPR1 had up to 75% grain chalkiness which was higher than in RD21, CNT1 and PTT1. By contrast, in the cool and rainy seasons, only 5 to 30% of the grain was chalky.

Furthermore, head rice yield of all varieties except RD21 was lower in the summer compared to the rainy season. Previous studies have suggested that high air temperature promotes chalkiness as well as drastically reducing head rice yield (Cooper *et al.*, 2008). The rice panicle is highly sensitive to high temperature especially in the ripening stage. It is possible that high temperature shortens the grain ripening period and this promotes grain chalkiness. Grain chalkiness is evident when there is a deficit of amyloplasts in the endosperm. Sato and Inaba (1976) hypothesized that insufficient supply of nutrients to the developing endosperm causes grain chalkiness. Hence, shortening the period of grain fill might limit the total nutrient loading delivered to the endosperm at maturity. Chalkiness is a serious problem for Thai farmers (Chapter 2) as it degrades the overall appearance of milled rice and generally results in lower head rice yield. This is due to chalky grain tending to be weaker and more prone to breakage during milling than translucent grain (Lisle *et al.*, 2000). Interestingly, farmers have some control over chalkiness as head rice yield is improved by N fertilizer management. Leesawatwong *et al*. (2005) shown that applying N fertilizer at grain filling increased the protein fraction in the peripheral region of the grain and the grain had increased resistance to forces produced during milling. Moreover, Nangju and De Datta (1970) reported that applying N fertilizer at the rate of 120 kg/ha improved milling quality of these chalky varieties; IR8, IR5, and Sigadis.

5.3 Nutritional quality and cropping season

The cropping season strongly influenced the accumulation of nutrients in the grain. In Chapter 2, all 4 varieties had greater grain Zn when grown in the cool season compared to the rainy and summer seasons, whereas grain Fe was higher in the summer season (Figure 5.2). The dilution effect could partly explain the high concentration of Zn in the cool season when compared to those of other seasons. Although the Zn concentration reflects the lower rice yield and 1000 filled grain weight, the grain Zn content in the cool season was higher than in the rainy and summer seasons. Therefore, some other factor influenced grain Zn other than seed weight. Probably, the ability of the rice plant to taken up soil Zn together with the ability to transport Zn to the grain also contribute to the higher grain Zn in the cool season. Grain Zn might original from Zn taken up by the roots after flowering (Jiang *et al.*, 2008) and/or Zn remobilized from leaves after flowering (Phattarakul *et al.*, 2012; Wissuwa *et al.*, 2008). Rice plants exude low molecular weight organic compounds such as citrate which facilitate the capture of Zn and its uptake into rice root cells. From there Zn is transported either as Zn^{2+} or in a bound form, for example with transporter proteins. Both Fe and Zn are complexed with nicotianamine (NA) (von Wirén *et al.*, 1999) as well as the ZIP family of metal transporter proteins in higher plants. Interestingly, the Zn transporter gene *OsZIP4* was detected exclusively for Zn translocation in rice (Ishimaru *et al.*, 2011; Ishimaru *et al.*, 2005).

A number of factors can influence the supply to the reproductive organs. Firstly, Fe is available when the soil is flooded. Secondly, high transpiration and respiration in the summer might promote root Fe^{2+} uptake from soil and transport to sinks as young tissues or the developing grain. However, under low Fe availability conditions, rice plants can take up Fe^{3+} by releasing phytosiderophores (PS) in the mugenic acid (MA) family which complex with Fe^{3+}. The Fe^{3+}-PS complexes are taken up into root cells by membrane transporters in the yellow-stripe (YSL) family (Bashir *et al.*, 2010; Ishimaru *et al.*, 2010; Kim and Guerinot, 2007) and transported symplastically until it is loaded in the xylem. The mechanism of subsequent xylem unloading to the symplast and reabsorbtion into the apoplast is unclear. However, Zn and Fe could be loaded directly from the xylem in the vascular bundle to the nucellar and aleurone cells. The developing grain is connected to the maternal plant by a single vascular bundle (Zhang *et al.*, 2007). This vascular bundle ends at the seed coat and is not connected to the endosperm or embryo. It is likely that nutrients are delivered to the maternal tissues surrounding the seed and effluxes apoplastically into the grain tissue (Waters and Sankaran, 2011).

From the results, most varieties had higher Fe concentration in brown rice grown in summer than in the rainy season, but this was not the case for white rice. This suggests that Fe is not easily transported into the endosperm. Saenchai *et al*. (2012) found that only a small proportion of Fe (1 to 3%) that was taken up by rice roots ended up in the grain. Prom-u-thai *et al*. (2007) suggested that the husk, which contains 24 to 47% of grain Fe could limit Fe transport to brown rice. Furthermore, the outer layer (pericarp, aleurone) of brown rice which are rich in protein bodies seem to be a major barrier for Fe transport into the endosperm. The removal of the embryo and aleurone layer during milling depletes most of the Fe, consequently white rice has very low concentration of Fe.

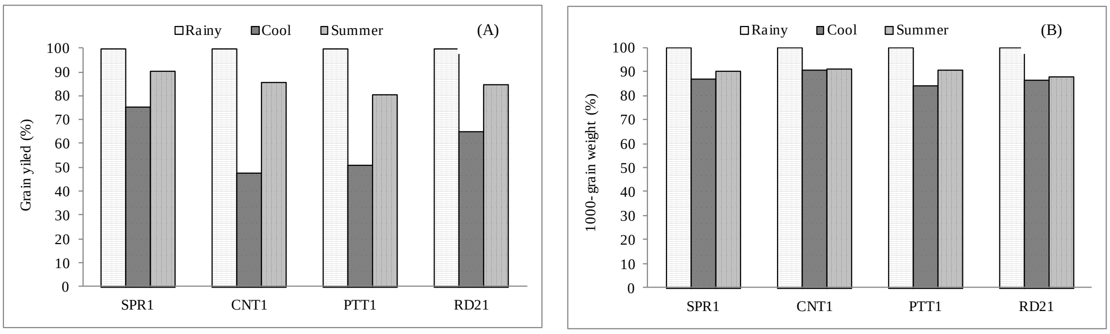

Figure 5.1 Relative value of (A) total grain yield and (B)1000 filled grain weight; of rice grown in three seasons (rainy, cool and summer).

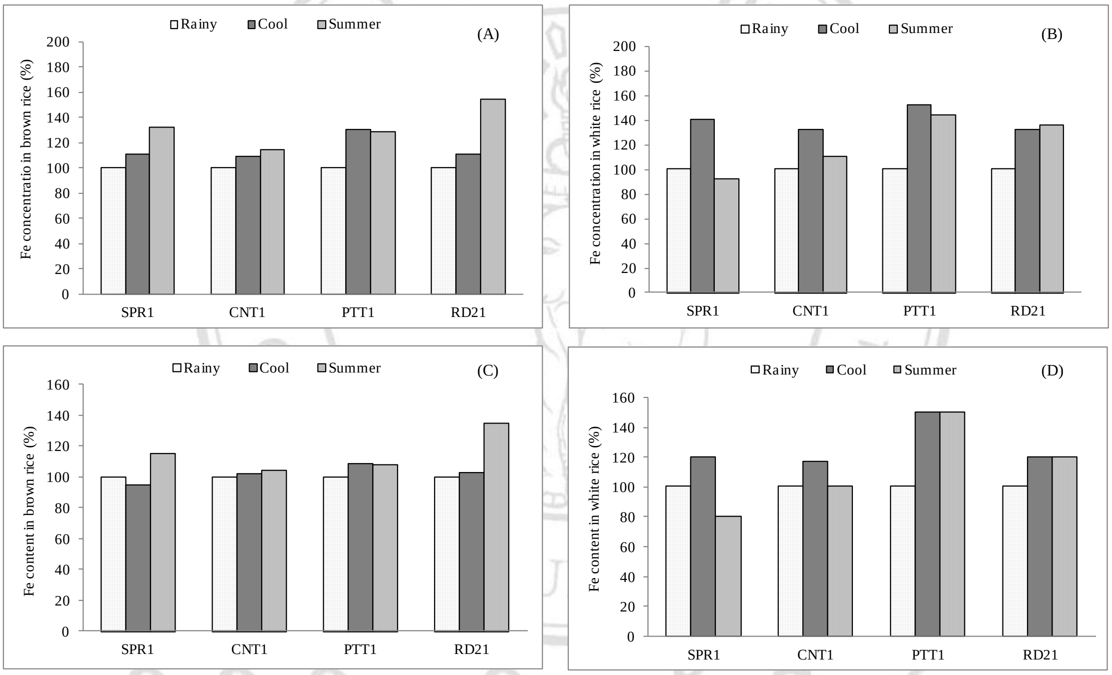

Figure 5.2 Relative value of grain Fe of rice grown in the cool and summer seasons compared to the rainy season: (A) Fe concentration in brown rice, (B) Fe concentration in white rice, (C) Fe content in brown rice and (D) Fe content in white rice.

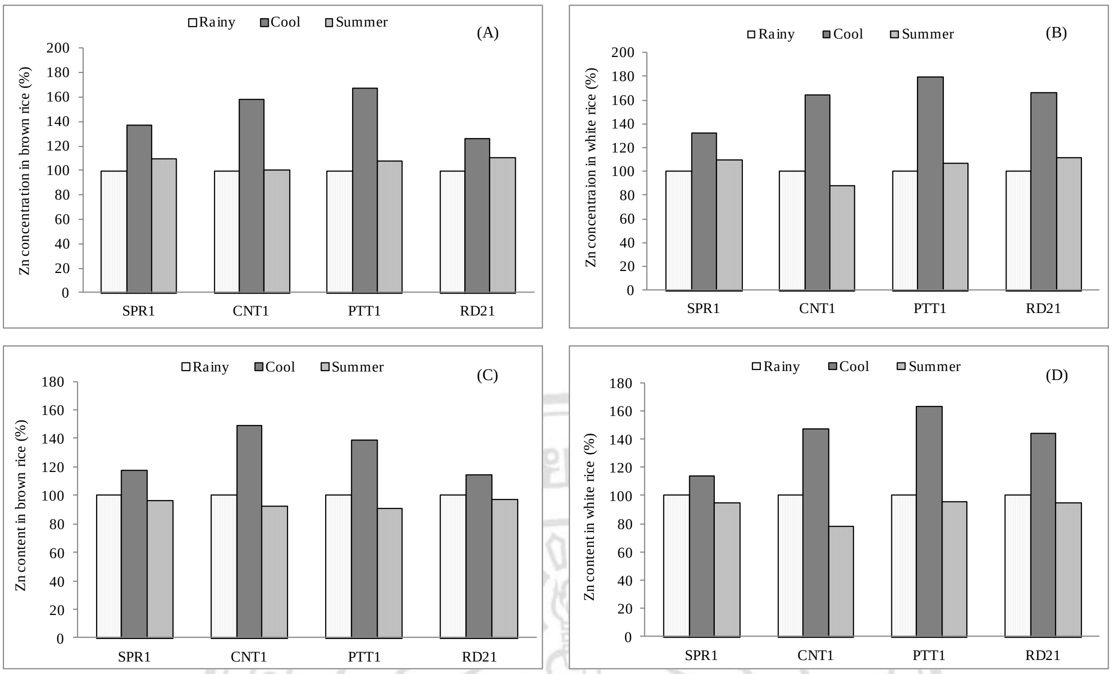

Figure 5.3 Relative value of grain Zn of rice grown in the cool and summer seasons compared to the rainy season: (A) Zn concentration in brown rice, (B) Zn concentration in white rice, (C) Zn content in brown rice and (D) Zn content in white rice.

5.4 Nutritional quality and foliar fertilizer

In recent years, foliar nutrient application has been reported to not only alleviate nutrient deficiencies in cereals such as wheat (Aciksoz *et al.*, 2011; Kutman *et al.*, 2010) but also to increase the Fe and Zn contents of rice grain (Phattarakul *et al.*, 2012; Yuan *et al.*, 2013). The timing of foliar nutrient application is an important factor determining the effectiveness of foliar fertilization. In Chapter 3, effects of foliar N, in the form of urea combined with Fe or Zn fertilizer were examined for grain nutrient qualities. Foliar fertilizers were applied to the panicles and leaves 7 days after flowering in the late afternoon to ensure that there was no deleterious effect on pollination. In the studied varieties, anther dehiscence occurred from 10.30 to 13.00 h Boonchuay *et al*. (2013) reported that applying Zn after flowering increased grain Zn by around 56%, which is in agreement with Phattarakul *et al*. (2012) who noted that foliar Zn applied after flowering under field conditions was more effective than application before flowering. In the present study Zn in both brown and white rice was increased by Zn application to the panicle and flag leaves. These results together with those of Phattarakul *et al*. (2012) and Boonchuay *et al*. (2013) confirmed that promoting Zn in the vegetative parts of rice by foliar Zn results in more Zn being re-translocated into the grain. In addition, Boonchuay *et al*. (2013) found a strong positive relationship between husk Zn and grain Zn. Another possible reason for enhanced Zn transport into the rice grain might relate to an increase in grain protein, especially in the early stage of grain development. Zinc is stored in protein bodies in the grain; therefore, increasing the density of protein bodies might increase the sink size for grain Zn. Generally, foliar fertilizer combining Zn with urea has shown positive effects on increasing grain N but a relationship between grain Zn and grain N has yet to be found. In conclusion, it seems that foliar Zn application is an effective way to improve the final Zn concentration in both brown and white rice.

Unlike Zn, applying foliar Fe to the rice panicle did improve the Fe concentration in brown rice and grain Fe was further increased when Fe was combined with urea. However, there was no improvement in the Fe in white rice. These appear to be strong barriers to Fe translocation and accumulation in the starchy endosperm. Further research should be conducted to understand this process. This knowledge can help underline strategies to increase rice grain Fe in the future.

It is worth noting that the effectiveness of foliar Zn or Fe application on grain Zn and Fe varied among rice genotypes. Although only 4 varieties were used in this thesis results a range of responses to foliar nutrient application; SPR1 and PTT1 showed a strong response to foliar Zn while CNT1 had only a slight response. It seems that the impact of foliar spraying on grain Zn or Fe can be maximized by selecting varieties that can absorb more nutrients and have a high ability to transport Zn or Fe into the grain.

Foliar application did improve nutrient quality especially grain Zn in both brown and white rice, however not the milling quality, as grain in the summer season still had lower percent head rice yield. Prom-u-thai *et al*. (2008a) reported that broken rice had higher Fe concentration than in whole grain. It would be useful to know whether broken rice always has higher nutrients than whole grain and the mechanism for this. In Chapter 4, it was found that the variation in Fe and Zn among grain fractions depended on the rice variety. In white rice, both the basal and distal fractions had higher Fe concentrations than the central fraction in most varieties, except in PSL1 which was higher only in the basal fraction. In contrast, the Zn concentration in white rice in all varieties was higher in the distal fraction than in the central fraction, which had similar Zn concentration as the basal fraction in all varieties, except SPR1 and CNT1 which had lower Zn concentration in the basal fraction than in the central fraction.

The translocation of nutrients into rice grain was reviewed by Oparka and Grates (1981) who suggested that nutrients are loaded into the grain thought the dorsal vascular stand and are then distributed to the tissue surrounding the grain and finally are effluxes into the grain apoplasm (Krishnan and Dayanandan, 2003). Mineral reserves in the grain are stored mostly in phytate-rich protein bodies in the embryo and aleurone layers of the grain, tissues that are important sinks for nutrient acumulation (Prom-u-thai *et al.*, 2008b). Most of the Fe and Zn in grain are stored in protein bodies which occur at a higher density in the embryo and aleurone layers than in the endosperm. It is possible that the distribution of protein bodies in the endosperm differs among rice varieites and grain parts, consequantly contributing of differences in grain Fe and Zn in different parts of the endosperm. Therefore, grain fractions that mainly make up broken rice as well as the differences in Fe and Zn among varieties would determine the level of nutrients in broken rice. In addition, broken aromatic rice produced from KDML105 and PTT1 can be expected to be richer in Fe than in the more expensive whole grain. This would be an advantage for rice consumers who face Fe and Zn deficiency problems and consume broken rice as stable food. Perhaps, in the future, the food industry could produce more food products from broken rice of high Fe and Zn content.

5.5 Genotypic variation of rice grain yield and quality

There was widely variation of rice grain quality among rice varieties and environment. As mentioned earlier, High temperature is a key factor inducing sterility and depressing yield. However, the effect seemed to be less serious for

Indica –Thai rice compared with the problem from low temperature. It has been documented in rice that the reproductive phase is the most sensitive to low temperature compared with the vegetative stage (Satake and Hayase, 1970; Satake and Koike, 1983). For example, low temperature dramatically reduced grain yield by inducing a lack of pollen for fertilization leading to reduced grain set (Satake and Hayase, 1970; Shimono *et al.*, 2007). Meanwhile, low temperature also causes reduction in culm and panicle length as well as incomplete panicle exsertion (Han *et al.*, 2002), and inhibition of pollen dehiscence and pollination leading to increased spikelet sterility (Chung, 1979). From the present study, grain yield was found to be lowest in all varieties in the cool season due to the unfertilized grains. Even though, there was no interaction between genotype and cropping season, the grain yield of SPR1 and RD21 was considerably higher than that of CNT1 and PTT1. One possible explanation for this is the cold tolerance variety had a dominant degree of panicle exertion during reproductive growth which has been suggested as an indicative character of genotype adaptability to cool temperatures (Takahashi, 1984).

Regarding their morphology, SPR1 and RD21 are long uppermost internode (peduncle) varieties while CNT1 and PTT1 are short peduncle varieties. When all varieties were subjected to low temperature, SPR1 and RD21 appeared to have a higher degree of panicle exsertion and produced more fertilized grain than CNT1 and PTT1. Possibly due to the shortened peduncle, the panicle and most spikelets of CNT1 and PTT1 were enclosed in the flag and this lead to the inhibition of pollen dehiscence and pollination. This is the reason why varieties with long peduncle such as SPR1 are recommended when damage from low temperature may occur during late vegetative growth in Thailand (Thai Rice Department, 2014).

In general, the head rice yields were reduced when grown in summer compared with the rainy and cool seasons. However, RD21 had consistently higher head rice yield than other varieties in all seasons, although the presence of grain chalkiness in this genotype was higher than the others in all seasons. This indicates that grain chalkiness may not always cause the problem of grain breakage. Leesawatwong *et al*. (2005) observed that head rice yield was correlated with the abundance of storage protein in the lateral regions of the grain, with a higher density of storage protein providing resistance to breakage during milling. In this study showed, RD21 had a higher grain N content than the other varieties. It is likely that the higher N accumulation in this variety results in a higher protein density in the lateral grain regions.

This is a wide variation in Fe and Zn concentrations in brown and white rice among different varieties (Gregorio, 2002; Prom-u-thai *et al.*, 2007; Prom-u-thai *et al.*, 2010; Sellappan *et al.*, 2009). However, some Thai commercial rice varieties have been reported having low concentrations of Fe and/or Zn (Saenchai *et al.*, 2012). Saenchai *et al*. (2012) reported that Thai commercial rice varieties such as CNT1, PTT1 and SPR1 had 8 to 10 mg Fe/kg and 17 to 24 mg Zn /kg in brown rice and these were depressed by milling by as much as 23 to 60% for Fe and 26 to 44% for Zn. However, from the results in chapter 2, grain Fe and Zn can be increased by growing the crop in different seasons and the degree of the increase was dependent on the rice variety. For example, grain Zn of CNT1 and PTT1 in both brown and white rice grown in the cool season was increased almost double when compared to the rainy and summer seasons. Moreover, PTT1 appeared to have higher Zn concentration in white rice than the other varieties in all seasons. Significant variation for grain Fe and Zn concentrations might possible due to variation in nutrient uptake efficiency and utilization among different genotypes. Uptake efficiency of plants may consist of an increased capacity to access soil nutrients and/or an increased capacity to transport nutrients across the plasma membrane, and uptake efficiency mechanisms might differ among rice genotypes. Differences in root development may play an important role in nutrient use efficiency among varieties. For example, nutrient efficient genotypes have longer and thinner roots and increased root surface area to absorption ions (Marschner, 1995). Besides, when subjected to nutrient deficiency such as Zn, the efficient varieties exudes long amount of low molecular weight organic compound such as citrate while can bind Zn facilitating uptake by plant roots (Marcherner., 1995).

In addition, the ability to transports ions into the grain differs among rice varieties. The results in chapter 3 showed that Fe and Zn remobilization from the leaves and panicle into grain differs among varieties. This might be due to differences in the ability to absorb foliar applied nutrients via leaf stomata as well as the ability to translocate Zn into the grain. The long-distance supply partways for Zn delivery to the grain may differ across varieties. For example, Wu *et al.* (2010) observed the remobilization of Zn from source tissues to the grain is an important source of grain Zn in some genotypes. However, Jiang *et al.* (2007) reported that root uptake was the predominant source of grain Zn loading into the grain. The current results revealed that there was genotypic difference in response to foliar Zn with CNT1 showing less response to foliar Zn than other varieties. This suggests that rice genotypes may differ in their response to foliar nutrient application when applied to increase grain nutritional quality. Therefore, there is a need for further research into increasing grain Fe and Zn, including selecting varieties with a high ability to absorb and translocate Fe and Zn into the grain.

Concentrations of Fe and Zn were different among grain fractions and it depended on variety. In white rice, both the basal and distal fractions had higher Fe concentration than the central in most varieties, except in PSL1 which was higher only in the basal fraction. In contrast, the Zn concentration in white rice in all varieties was higher in the distal than the central fraction, which had similar Zn concentration as the basal fraction in most varieties. The reason for the differential distributed of Zn along the grain remains to the elucidated. Perhaps some nutrients are preferentially unloaded at the vain ending and other are unloaded more uniformly along the length of the dorsal vascular bundle.

5.6 General conclusion

This study concluded that rice grain qualities were influenced by environmental conditions and it varied among varieties. The cropping season affected grain yield and quality, mainly due to difference in temperature at flowering. Low temperature in the cool season severely reduced grain yield. However, grain nutritional quality such as concentrations of N, P and Zn in the grain, was increased. High temperature in summer also impacted grain quality by increasing grain chalkiness, decreasing head rice yield and increasing GT.

Improving rice grain quality by foliar Fe and Zn application had been successful in terms of improving grain nutritional quality but urea application can promote rice grain Fe but not grain Zn. Foliar application of Fe increased brown rice Fe concentration and this was further improved when Fe was combined with urea. Foliar Zn alone or combined with urea significantly increased the Zn concentration of both brown and white rice. However, foliar Fe and Zn reduced the milling quality of grain but the depression of milling quality was reduced when foliar Zn combined with urea.

There was variation in the Fe and Zn concentrations along the grain length both in brown and white rice. The Fe and Zn concentrations of most varieties were higher in the basal and/or distal fractions than the middle region depending on the variety. Therefore, broken rice can sometimes have higher Fe and Zn concentrations than the whole grain rice depending on the proportion of grain fractions that make up most of the broken rice.

5.7 Future research

1. The present study identified strong seasonal responses in grain Fe and Zn. The concentration of Fe in the rice grain was increased in the summer crop, while the Zn concentration was increased in the cool crop. These finding should be extended to a broader range of the effect of genotypes. These studies should also investigate the effect temperature on Fe and Zn transport and accumulation in the grain filling. Ideally, do this, the experiment should be carried out in growth chambers where temperature, light and humidity are controlled.

2. The data obtain from this study suggested that applying Zn or Fe fertilizer by foliar spraying could improve grain Zn in white rice and Fe in brown rice. The lack of Fe transported into white rice should be explored further by:

    - Modifying the rate of Fe fertilizer,
    - Using different forms of Fe such as Fe(II)-EDTA, Fe(III)-HEDTA, Fe-NA or Fe-AA, and

- Testing the timing of foliar applied at 5, 10, 15 and 20 days after flowering.

3. Further in depth study is require to investigate metal transporters, distribution of metal-binding storage compounds and the processes that govern the transport of Fe into the endosperm and other parts of the grain. This would help to understand the pathway and timing of Fe and Zn accumulation in the grain. Progress is likely to be make by molecular and physiological/biochemical tools.