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Levels of nitrogen and potassium [N:K] fertilizer combinations in commercial rice production system

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Rice grain chalkiness can reduce crop quality resulting in lower grades and returns. It is as a consequence of loose starch and protein particles packing during grain filling and maturation, and may be due to nutritional, biochemical, physiological and environmental conditions particularly in varieties with high inheritable chalkiness. This study set out to evaluate the response to varying levels of nitrogen and potassium [N:K] fertilizer combinations and its time of application [TOA] on the partitioning of assimilates and their effect on grain filling and chalkiness in a commercial rice production system. The results indicate that while the N level increased within a particular K level, the crop maintained a similar rate of photosynthesis and dry matter production. The K which is involved in active translocation manifested its effect later in the partitioning after 14WAS, and during grain filling. At the N:K ratio of 2:1, the % chalkiness was at an acceptable level (<2%), and the plant tissue analysis N:K was close to 1:1. Chalkiness is an agronomic problem that is best managed in susceptible varieties through adequate crop nutrition using the simple N:K ratio. The study confirmed that when N:K was applied at a 2:1 ratio, grain yield was the highest and chalkiness at its lowest

Key words: Grain filling, chalkiness, net assimilation rate, partitioning of assimilates, potassium fertilizer.

INTRODUCTION

Grain chalkiness in rice is a highly undesirable industrial quality trait in the marketing and consumption, and influences the commercial value. Chalky kernels range from 1 to 15% [U.S. No. 1 to 6, respectively] for long rice grains (USDA, 2005). These grains break more readily than translucent ones (Bridgemohan, 1997), thus reducing whole grain head rice yields (HRY). Currently, world markets do not accept any rice which has more than 2% chalky rice.

Chalkiness is characterized by opaque areas in various parts of the rice kernel which occurs when starch and protein particles become loosely packed or incompletely filled within the endosperm during grain maturation (Ebron, 2013). This may be influenced by a combination of factors including, physical, biochemical, physiological, water quality and availability (Chen et al., 2012), temperature (Shen et al., 1997), crop nutrition (Mingli and Yonggui, 2005; Sun et al., 2014), cultivar genetics

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(Yamakawa et al., 2007) and production environment and cropping systems (Carlos et al., 2008).

The molecular basis of this trait is poorly understood but the quantitative trait locus (QTL) is identified as *Chalk*. This encodes a vacuolar H⁺-translocating pyrophosphatase (V-PPase) with inorganic pyrophosphate (PPi) hydrolysis and H⁺-translocation activity and has been shown to influence grain chalkiness, which also affects HRY (Yibo et al., 2014).

Ishimaru et al. (2009) reported that high-temperature stress during grain ripening causes induces loose packing of the amyloplasts. Shen et al., (1997) found that with high temperature treatment, peaks of materials synthesis and enzyme activity in endosperm were earlier than those of proper temperature treatment. This affected the sequence and pattern of changes of materials and enzymes in endosperm during normal grain filling, and further intensified chalkiness. Molecular mapping (Chandusingh et al., 2013) and magnetic resonance images (Ishimaru et al., 2009) of the early stage caryopses in the high-temperature condition confirmed loose packing of amyloplasts in the chalky part of the grain. Rice breeding programs usually discard chalkiness lines, but it can still manifest over time in some environments.

Zhou et al. (2009) reported that chalkiness is related to "source-sink" of rice, dynamics of grain filling, and biosynthesis and accumulation of starch in endosperm. It is a complicated quantitative trait, which is controlled by maternal, endosperm and cytoplasmic effects. They noted that there are some stable QTL for rice chalkiness on many rice chromosomes, which have an impact on starch synthesis, starch metabolism, and fruit development. However, the manipulative network and formative mechanisms of rice chalkiness remain unclear, and the decrease of rice chalkiness is a major aim in rice quality breeding.

Sun et al. (2014) showed that different water-nitrogen management patterns and P:K fertilizer combined application significantly affect rice quality, nutrient absorption and distribution and grain HRY. They found that amylose and protein contents under different water - nitrogen management patterns are higher than those of different P-K fertilizer combined applications, while the % chalkiness and gel consistencies are the opposite.

Motoki et al. (2013) confirmed that chalky grains are caused by high temperature stress at the ripening stage. Chikako et al. (2013) showed that deep-flood treatment enhanced N uptake, and consequently photosynthetic activity, resulting in the reduction of chalky grain formation. Shenggang et al. (2013) surmised that there is a role for plant growth regulators in grain yield, quality and antioxidant enzyme activities, but little is known about.

This study set out to evaluate the response to varying levels of nitrogen and potassium [N:K] fertilizer combinations and its time of application (TOA) on the

partitioning of assimilates and their effect on grain filling and chalkiness in a commercial rice production system.

MATERIALS AND METHODS

Two commercial field studies were conducted the Caroni Rice Project [CRP] and one pot study at the Centre Bioscience, Agriculture and Food Technology, Waterloo Research Campus, University of Trinidad and Tobago.

The soil type was the Frederick soil series loam with an average CEC of 4.8 meq/100 g; a pH (water soil solution) of 5.25; N at 0.313 % ; P of 7.5 to 11.9 mg.kg⁻¹ ; K at 124 to 160 mg.kg⁻¹, and a sand, silt, and clay content of 61.0, 14.5, and 24.5%, respectively, in the upper 0.5 m soil. The pot trials were established using the same soil type as that of the commercial fields. For this purpose, the soil was pulverized and sieved to remove all debris, and solarized to kill weed seeds.

The seed material used was var. Oryzica 1 which is the major commercial variety. This is a high yielding variety and is considered to have low percentage chalkiness. However, over the last six years, this has varied from 2.5 to 20% depending on the field conditions, time of the year, rainfall patterns, and the timeliness of the fertilizer application. The seeds were broadcasted by aerial application at seeding rate of 50 kg seed.ha⁻¹. The crop was cultivated under rain fed conditions of the wet season (June to October) and there was no need for supplemental irrigation.

Aerial application for the seed, fertilizer and pesticides were done using a Cessna AJ Truck A188B aircraft which is calibrated to deliver the appropriate rates needed for the study. All applications were done during the hours of 6.30 to 8.30 am when the wind speed was less than 10 knots h⁻¹ flying at 90 knots h⁻¹ at 8 to 10 m above ground level, and average daily temperatures between 22 to 25°C.

The crop was subjected to all the standard agronomic and estate management practices for commercial fields with respect to land preparation, sowing, water management and weed control. All treatments received a standard dressing of phosphorous (P₂O₅) at a rate of 58 kg.ha⁻¹ as Triple Super Phosphate (TSP) at the time of the first nitrogen application. The normal agronomic practices of insect control [cypermethrin 225 CE (1l/ha) and fipronil (1l.ha⁻¹)] and diseases control [Difenoconazole] were conducted. Weed control was done using propanil at 3.0l.ha⁻¹ at 2 weeks after sowing (WAS) and a mixture of Oxadiazon (1.25 ka. ai.ha⁻¹) and Bromoxyl + 2,4D ester (1.0 ai.l.ha⁻¹) at 7 WAS. The field crop was harvested by combine harvester and plot yield and HRY recorded.

Study 1

This field experiment was established at CRP on the entire field of Nos. 003 and 005 which were 12 ha each. The fertilizer treatments were Nitrogen at 2 rates (75 and 90 kg.ha⁻¹ (applied as split TOA at 4.5 and 9.0 weeks after sowing (WAS) and Potassium at 3 rates (0, 50, and 90 kg.ha⁻¹) at the similar TOA (Table 1). The trial was laid out as a split plot, with nitrogen as the main plots and potassium in the sub-plots, with time of application randomized within sub-plots and replicated three times. The experimental plots were 12 m in width by 500 m in length and were normally manually harvested, whilst the remainder of the field was mechanized.

Study 2

This field experiment was conducted the next year following study 1 at CRP in field 205, with experimental plots sizes of 10 m × 50 m. It was laid out according to split plot design with 4 main plots and 3

Table 1. Treatment combinations of the N:K fertilizer [kg.ha⁻¹].

Time of application [TOA] WAS	Potassium [K] rates kg.ha ⁻¹	Nitrogen [N] rates kg.ha ⁻¹
0	K ₀ (0)	N ₁ (75)
4.5	K ₁ (50) K ₂ (50)	N ₂ (90)
9.0	K ₃ (90) K ₄ (90)	

Table 2. Treatment combinations of the N:K fertilizer [kg.ha⁻¹].

Potassium [K] rates kg.ha ⁻¹ at TOA (6 and 9 WAS)	Nitrogen (N) rates kg.ha ⁻¹ at TOA (3, 6 and 9 WAS)
K ₁ (45)	N ₁ (30)
K ₂ (60)	N ₂ (45)
K ₃ (90)	N ₃ (60)
	N ₄ (90)

subplots, with 3 replicates. The N:K treatments were Main Plot: N (30, 45, 60 and 90 kg N.ha⁻¹) in split TOA (3,6,9 WAS) and Subplot: Muriate of Potash (45, 60, and 90 kg K₂O.ha⁻¹) which was applied only at 6 and 9 WAS in a split TOA (Table 2).

Study 3

The pot experiment was conducted under full sunlight conditions and established in plastic drums filled with 50 L of the solarized soil. The N:K fertilizer treatments were nitrogen at 4 rates [50, 75, 100 and 125 kg.ha⁻¹] and potassium fixed at 45 kg.ha⁻¹ using similar TOA as Study 2. The pots were placed 30 cm × 30 cm apart and water level kept flooded above field capacity during growth and grain filling period. Leaf growth and plant dry matter production were monitored at 10 days intervals until early panicle emergence.

In all studies, leaf tissue and soil samples were analyzed to determine the levels of N, P and K. At the times of aerial application of fertilizer, containers (30 × 45 cm) were randomly distributed throughout the experiment field to monitor the aerial discharge and quantity of fertilizer reaching the crop. Grain yield for each treatment was computed from five 10m² quadrant. The data collected included tillering ability, filled spikelets, yield components and grain yield. The N was determined by means of the of a Coleman Junior 11A Linear Absorbance Spectrophotometer and the K by Flame Photometry using a Corning Eel Flame Photometer.

Plants were harvested for biomass measurements starting from 20 days after emergence (DAE) at intervals of 10 days until harvest. At each harvest, plants were separated into leaf laminae, petioles, tillers, and roots and dried in a forced-air oven at 65°C for 72 h (to constant weight) for dry matter determination. Best fit polynomial regression curves were used for growth analysis between two successive harvest periods [HP]. Relative growth rate (RGR), net assimilation rate (NAR), specific leaf area (SLA), leaf Area (LA), leaf area ratio (LAR), leaf weight fraction (LWF), shoot dry matter as described by Hunt et al. (2002). Kernel chalkiness determined by visual rating of the chalky proportion of the grain and is used to measure chalkiness based on the standard Evaluation System SES scale presented below: Select, segregate and weigh the chalky

grains (SES Scale 9). Determine the % chalky grain using the equation:

Scale	% area of chalkiness
1	less than 10
5	10 - 20
9	more than 20

$$\% \text{ Chalky grain} = \frac{\text{Weight of chalky grains}}{\text{Weight of milled rice}} \times 100$$

Air temperature, rainfall, and solar radiation during the experiments were calculated with an automated weather station (Davis Instruments, California) located within the station compound and at close proximity to commercial fields. Light interception was determined during the morning (10.00 to 11.00 am) by measuring the radiation above the canopy, and at soil level using an integrated quantum sensor (Model LJ – 185B Quantum Radiometer/Photometer, LICOR, Inc, Lincoln, NE 68504). Leaf area was measured using an area meter (Type AAM – 5 Hayashi Denko Co. Ltd. Japan). All data were analyses as multiple regression analysis using Minitab Statistical package.

RESULTS AND DISCUSSION

Study 1

The results (Table 1) indicated that the varying rates and time of application of the N:K combinations had no significant effect on final plant height at harvest (82.1 ± 0.823 cm), and tiller number (240.4 ± 7.66%). There appeared to be no trend on the productive particle (%). In all cases, regardless of the N:K combination, the % chalkiness was in excess of the acceptable industrial standard (Table 1). At the lower nitrogen N₁ rate (75 kgN.ha⁻¹), it was observed that by increasing the potassium

Table 3. The response of rice variety Oryzica 1 to varying rates and time of application of nitrogen and potassium fertilizer.

Treatment combination	Plant height	Tiller Nos. (Nos. on ⁻²) (cm)	% Productive panicle	Chalkiness (%)	Yield (t.ha ⁻¹)
N ₁ K ₀	84	280	86	11.8	2.6
N ₁ K ₁	83	208	95	13.0	2.6
N ₁ K ₂	79	247	93	15.4	3.1
N ₁ K ₃	84	256	91	10.2	3.7
N ₁ K ₄	81	226	94	10.6	2.4
N ₂ K ₀	84	254	90	15.6	2.8
N ₂ K ₁	84	251	88	13.6	2.7
N ₂ K ₂	85	205	87	17.0	2.4
N ₂ K ₃	78	256	93	15.0	2.3
N ₂ K ₄	79	221	89	9.0	2.1
Mean	82.1	240.4	90.6	13.1	2.6
S.E [±]	0.82	7.66	0.98	0.85	0.15

Table 4. The effect of various combinations of nitrogen and potassium fertilizer on the development and yield of rice variety Oryzica 1 1996.

Treatment combination	Tiller Nos. (Nos. on ⁻²) (cm)	% Productive panicle	Chalkiness (%)
N ₁ K ₁	212	100	3.3
N ₂ K ₁	216	100	5.3
N ₃ K ₁	304	96	5.0
N ₄ K ₁	278	96	3.0
N ₁ K ₂	275	100	4.0
N ₂ K ₂	333	100	5.3
N ₃ K ₂	414	99	3.7
N ₄ K ₂	293	100	4.0
N ₁ K ₃	348	100	2.0
N ₂ K ₃	440	100	4.3
N ₃ K ₃	491	96	3.3
N ₄ K ₃	436	100	6.7
Mean	336.7	98.9	4.13
S.E[±]	26.2	0.514	0.36

rate, the rice grain yield increased linearly (Equation 1).

$$Y_{\text{Grain yield}} = 2.05 + 0.38 \text{ N:K } R^2 = 0.88 \quad (1)$$

However, an opposite trend was observed at the higher N₂ rate (90 kg N. ha⁻¹), as the K₂O rate increased, the grain yield decreased linearly (Equation 2).

$$Y_{\text{Grain yield}} = 2.85 - 0.19 \text{ N:K } R^2 = 0.96 \quad (2)$$

It was difficult to partition between the time of application between 4.5 and 9.0 WAS, compared to rates. However, it appeared that N₁K₃ (75 kg N + 90 kg K₂O at 4.5 WAS) produced the highest yield and was above the normal

yield of 3.5 t. ha⁻¹ (Table 3).

Study 2

The key difference between experiments 2 and 1 was that the nitrogen fertilizer was applied at 4 rates in three split applications, and the K₂O at three rates, and split into two TOA. It is to note that the first N application was at 3 WAS and applied single with no K₂O₅ at this time. The results showed that increasing the N rates (Table 4 and 5) at 45 kg K₂O ha⁻¹ increased grain yield linearly (Equation 3)

$$Y_{\text{Grain yield}} = 1.9 + 0.4 \text{ N:K } R^2 = 0.83 \quad (3)$$

Table 5. The effect of various combinations of Nitrogen and Potassium fertilizer on the development and yield of rice variety Oryzica 1 1996.

Nitrogen kg ha ⁻¹	Rates of fertilizer		
	Potassium [K ₂ O] kg ha ⁻¹		
	45	60	90
	Rice grain yield (t.ha ⁻¹)		
30	2.5	3.4	2.5
45	2.5	3.2	3.0
60	2.9	2.9	3.2
90	3.7	2.4	3.0
Equations	3	4	

Table 6. Leaf tissue and soil analysis of the of the varying N:K treatment combinations at 9 WAS.

N*K fertilizer rates	NPK content (%)					
	Leaf tissue			Soil analysis		
	N	P	K	P	K	pH
N ₁ K ₁	3.27	0.30	2.31	10.00	129.33	5.20
N ₂ K ₁	3.02	0.31	2.31	10.67	140.00	5.18
N ₃ K ₁	3.17	0.28	2.19	9.67	134.67	5.25
N ₄ K ₁	2.93	0.31	2.15	8.67	143.33	5.32
N ₁ K ₂	3.15	0.31	2.25	10.00	129.33	5.15
N ₂ K ₂	2.72	0.29	2.21	8.33	128.00	5.17
N ₃ K ₂	3.22	0.29	2.20	9.33	177.33	5.33
N ₄ K ₂	3.45	0.27	2.15	10.00	120.00	5.22
N ₁ K ₃	2.83	0.29	2.05	9.33	117.33	5.17
N ₂ K ₃	2.87	0.25	2.19	9.00	125.33	5.23
N ₃ K ₃	3.23	0.31	2.49	10.67	144.00	5.20
N ₄ K ₃	2.90	0.30	2.13	9.00	152.00	5.18

However, the opposite occurred when the rate increased to 60 kg (K₂O ha⁻¹) and it decreased linearly (Equation 4).

$$Y_{\text{Grain yield}} = 3.8 - 0.33 N^*K \quad R^2 = 95.9\% \quad (4)$$

Grain yield peaked at the highest nitrogen rate (90 kg N₂ ha⁻¹) and lowest potassium (45 kg K₂O. ha⁻¹) producing 3.7 t. ha⁻¹ of quality rice grains with an acceptable chalkiness of 2.0% at the split fertilizer TOA at 6 and 9 WAS (Tables 4 and 5). This compared to the highest grain yield in Trial 1 which had a similar yield (3.7 t. ha⁻¹) at 70 kg N. ha⁻¹ plus 50 kg (K₂O) at a single application [4.5 WAS], but a chalkiness of 10.2%.

The difference in both trials attaining at high of 3.7 t. ha⁻¹ was that the treatment with the lowest chalkiness had both nitrogen and potassium as split applications. That is, a single application of nitrogen was made at 3 WAS, and the other 2 application of N coincided with the potassium application at later dates of 6 and 9 WAS, respectively. The results suggested that splitting the nitrogen

application at equal intervals TOA (3, 6, 9 WAS) , and using a lower rate in a split application (6 and 9 WAS) produced the highest yield, and the lowest chalkiness. Further, the crop was making better use of the Nitrogen prior to panicle initiation at 9 WAS. At that treatment (Table 6), the tissue analysis, indicated that the % NPK was adequate (Table 7), and the crop was making better uptake and use of the soil Phosphorous.

Study 3

This pot trial was established to validate the TOA of the N:K combinations in experiments 1 and 2, and determine the influence on the sink-source relationship, and the subsequent partitioning of assimilates and grain filling and chalkiness in rice.

The partitioning of assimilates analyzed through growth analysis revealed that the varying N:K combinations treatment had no effect over the various harvest period

Table 7. Levels of nutrient adequacy from tissue analysis for rice variety Oryzica 1 (Shand, 1997).

Nutrient	Levels of adequacy		
	Deficient	Critical	Adequate
N	<2.9	2.9	>3.0
P	<0.12	0.13 to 0.21	>0.22
K	<0.84	0.85 to 1.96	>1.97

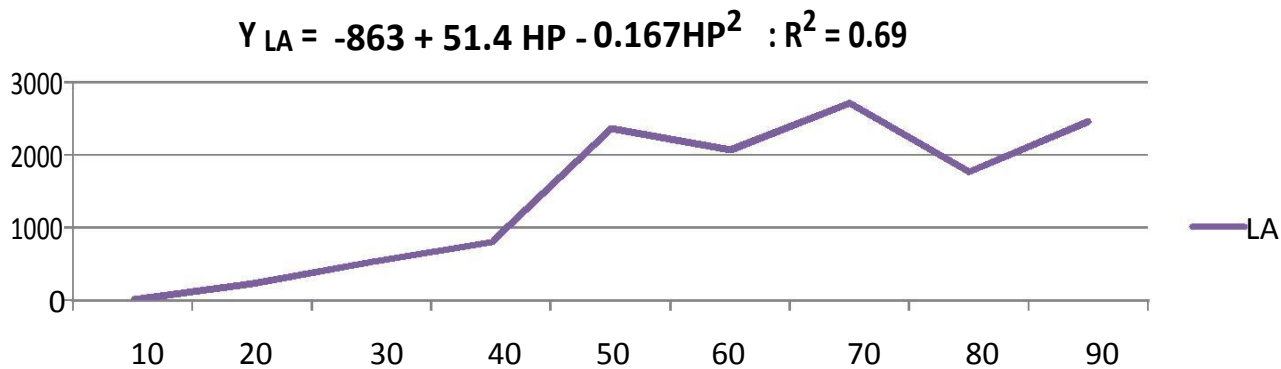


Figure 1. Leaf area (cm²) of rice (var. Orizica1) under varying levels and time of application of N:F fertilizer.

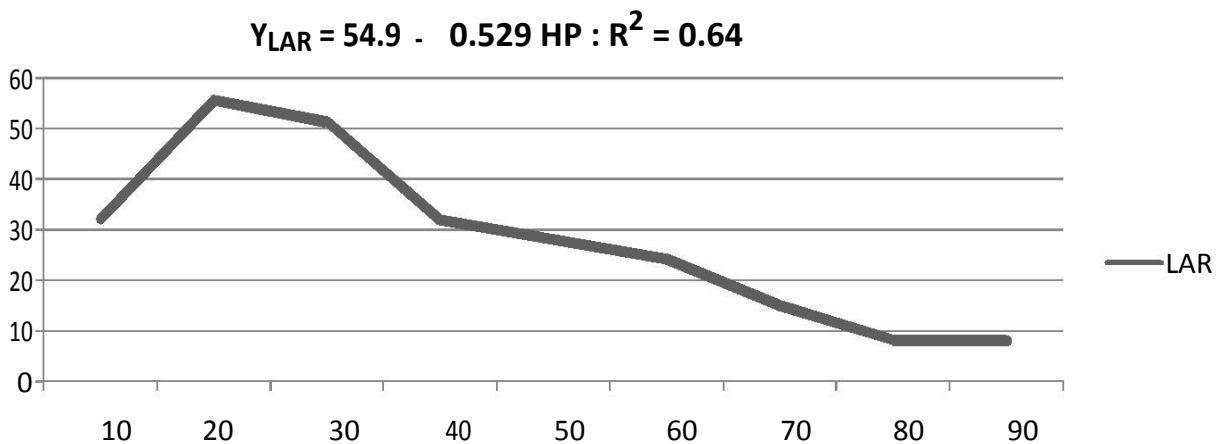


Figure 2. Leaf area ratio [LAR (g/cm²/day⁻¹)] of rice (var. Orizica1) under varying levels and time of application of N:F fertilizer.

[HP] until flowering at 95 DAS. It showed that the leaf area (Figure 1) and LAI (Figure 4) exhibited the usual quadratic response over HP. Similarly, the LAR (Figure 2) and LWF (Figure 3) declined linearly and were unaffected. The crop reached its maximum leaf area index [3.85] at 70 to 80 days after sowing. Both the RGR (Figure 5) and NAR (Figure 6) showed no treatment effect over the HP, and displayed quadratic responses.

Like the other two studies, the N*K combinations did not influence the number of panicles or the tiller number /stool, but the number of grains and grain yield increased linearly with increasing levels of nitrogen (Table 8). The acceptable level of chalkiness [2%] was attained at the 100 kg N : 45 kg K], and increased with lower or higher levels of N to the fixed K.

Whilst the N fertilizer level was increased at the fixed K

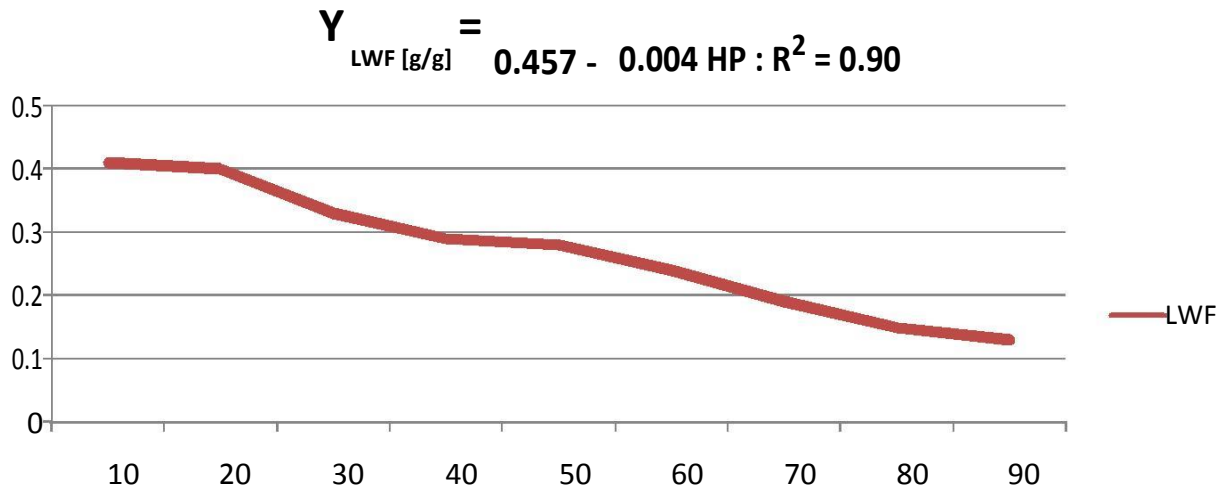


Figure 3. The Leaf Weight Fraction [LWF (g/g)] of rice (var. Orizica1) under varying levels and time of application of N:F fertilizer.

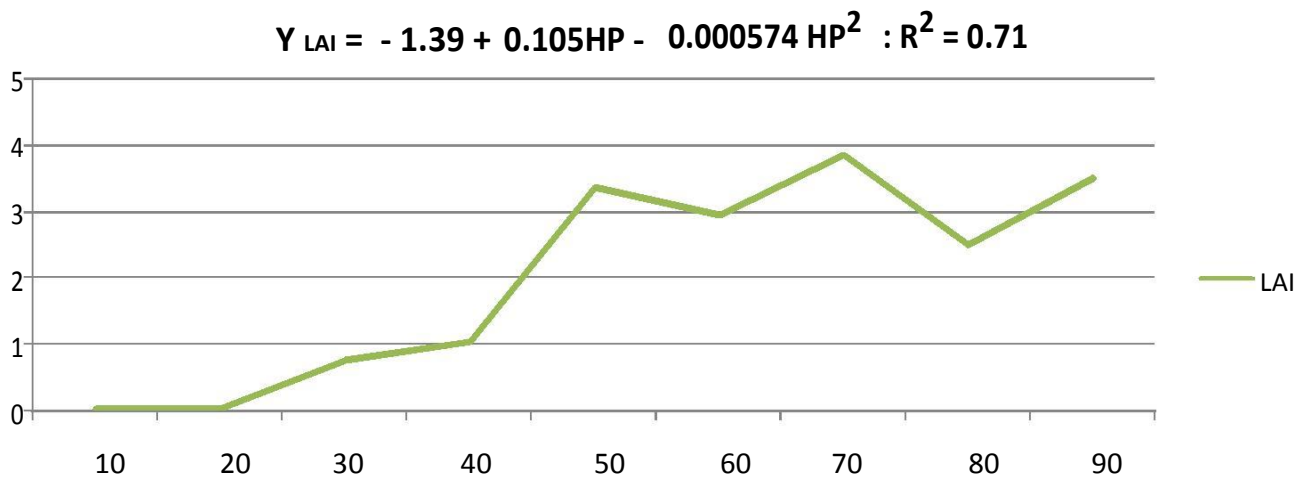


Figure 4. The leaf area index [LAI] of rice (var. Orizica1) under varying levels and time of application of N:F fertilizer.

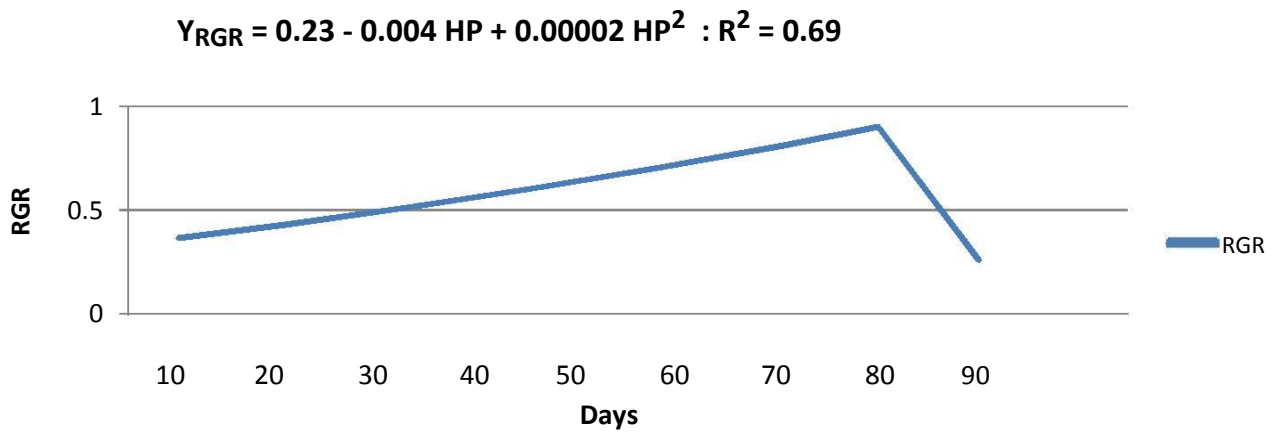


Figure 5. The Relative growth rate [RGR (g.m².day⁻¹)] of rice (var. Orizica1) under varying levels and time of application of N:F fertilizer.

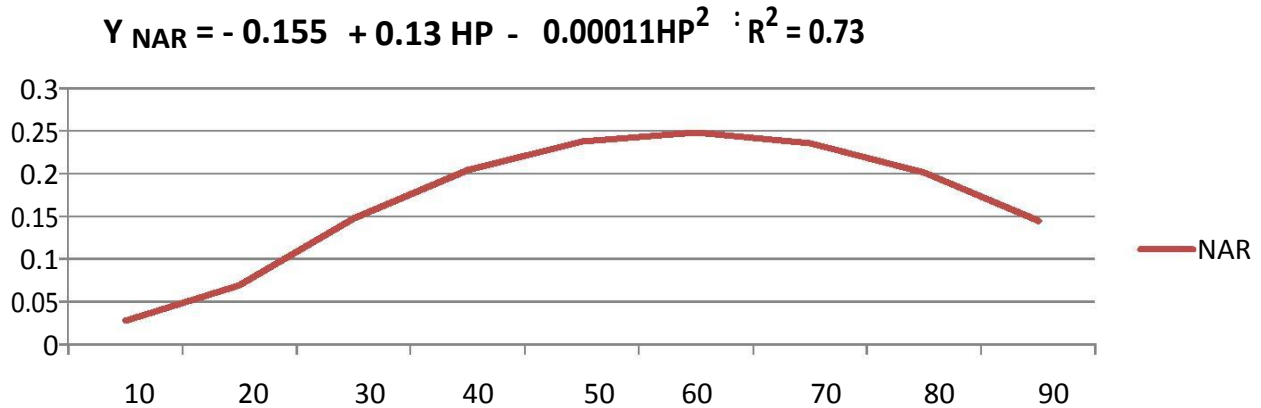


Figure 6. The net assimilation rate [NAR (g.m².day⁻¹)] of rice (var. Orizica1) under varying levels and time of application of N:F fertilizer.

Table 8. The effect of varying N:K treatment combinations on the reproductive capacity of rice.

Nitrogen kg ha ⁻¹	No. tillers / stool	Nos. panicle / stool	Chalkiness %	Number of grain/stool	Grain yield / stool
50	46	32	3	2872	78.1
75	43	37	4.7	2902	78.2
100	28	37	1.9	3056	83.1
125	42	36	6.1	3570	97.1
Mean (S.E)	42 (2.07)	34.1 (1.16)	-	3100 (185)	Y=37.5+6.12 N:K+21.0 HP; R ² = 88.6%

level, the crop maintained a similar rate of photosynthesis and dry matter production. The K which is involved in active translocation manifested its effect later in the partitioning of assimilates after 90 DAS, and during grain filling. N is significant in photosynthesis and production of assimilates, but K is the key element in determining the efficiency of the 'sink- source 'relationship and the prevention of the loose packing of the starch and protein particles (Chikako et al., 2013).

At this period, the evidence showed that when the N:K ratio was 2:1, the % chalkiness was at an acceptable level (Bridgemohan, 1997). However, above the ratio, the number of grains appeared to increase (not significant), and the grain yield increased linearly (Table 6), but also the % chalkiness reached in excess of 6%. Mingli and Yonggui (2005) found that the grain yield in rice can be improved applying a urea solution during ripening period. However, while the amylose content was improved and gel consistency was extended, the chalkiness was increased.

Chalkiness is an agronomic problem that is best managed in susceptible varieties through adequate crop nutrition using the simple N:K ratio. The study confirmed that when N:K was applied at a 2:1 ratio, grain yield was the highest and chalkiness at its lowest, and that the plant

tissue analysis N: K was close to 1:1. Since most of the experiments were conducted in the wet season, it is suggested that the growing environment could have impacted in kernel chalkiness, which can be attributed to nighttime air temperature during grain filling. It is also accepted that there are some susceptible chalky cultivars, but the selected var. which is known to be of good quality has succumbed to variations in N:K nutrition in the wet season.

Conflict of Interest

The authors have not declared any conflict of interest.

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