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Nitrogen use efficiency is associated with chlorophyll content in Brazilian spring wheat

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Abstract

The use of wheat cultivars with high nitrogen use efficiency (NUE) is essential to reduce production costs and minimize environmental contamination through nitrogen losses. Despite several investigations reported worldwide, NUE still has not been studied in Brazilian wheat varieties. The objective of this study was to evaluate the general and specific combining ability (GCA and SCA) of agronomic traits associated with NUE components in a diallel cross. Six wheat parents and fifteen F_2 populations were planted in the field in a randomized block design with three replications. The relative chlorophyll content was measured in flag leaves at the post-anthesis stage, while agronomic traits and NUE components were evaluated at physiological maturity. Additive and non-additive genetic effects were identified controlling NUE components. The parents, Mirante, Valente, and BRS Tangará, showed high values of GCA for NUE components. The chlorophyll B index was significantly correlated with grain yield (0.49^*), thousand kernel weight (0.44^*), and NUEg (0.50^*), indicating that chlorophyll B index can be used as an effective selection criterion in early segregating generations. To our knowledge, this is the first report of genetic variability of nitrogen use efficiency in Brazilian spring wheat.

Keywords: Combining ability; diallel cross; F_2 population; grain protein concentration; *Triticum aestivum* L.
Abbreviations: BNC_biomass nitrogen concentration; Chl_A_Chlorophyll A index; Chl_B_Chlorophyll B index; Chl_{A+B}_Total chlorophyll content index; CV_coefficient of variation; DF_degrees of freedom; GCA_general combining ability; GNC_grain nitrogen concentration; GPC_grain protein concentration; GY_grain yield; H^2_broad sense heritability; KPS_kernels per spike; NHI_nitrogen harvest index; NSP_number of spikes per plant; NUEg_nitrogen use efficiency by grains; PPE_production protein efficiency; SA_selective accuracy; SCA_specific combining ability; TKW_thousand kernel weight; TW_test weight.

Introduction

Nitrogen is the most required nutrient by wheat crops: it plays an important role in biochemical processes as part of protein structures, enzymes, nucleic acids, and chlorophyll molecules (Singh et al., 2012; Kong et al., 2013). Hence, it is essential to effectively manage this nutrient in order to achieve a high wheat yield. Recent studies reported that the use of nitrogen fertilizers have increased sevenfold since 1960 and its demand continues to grow (Ryan et al., 2012). Thus, nitrogen use efficiency (NUE) as a trait should be considered when selecting wheat varieties, based on its role in increasing agronomic and economic efficiency as well as minimizing environmental contamination. NUE can be defined as the ratio of grain yield or biomass by the nitrogen supply available in the soil (Moll et al., 1982) and the study of NUE components separately allow obtaining the most accurate information (Salvagiotti et al., 2009). Several surveys have reported that NUE values are less than 60% in wheat (Barraclough et al., 2010; Gorny et al., 2011; Hawkesford, 2012). Inefficient cultivars promote the loss of nitrogen by volatilization and leaching, negatively affecting the environment (Gaju et al., 2011; Kong et al., 2013). As a result, finding genotypes with high NUE values is very important because they enable cultivation in situations where this nutrient is expensive or when the application is limited due to unfavorable weather conditions. Identifying agronomic traits associated with NUE indexes and characterizing this genetic variability generates useful information for wheat breeders (Barraclough et al., 2010; Gaju et al., 2011). The nitrogen harvest index (NHI) stands out among the NUE components, because it indicates the translocation rate of nitrogen from the straw to the grains; normally it tends to vary from 69% to 98% (Barraclough et al., 2010; Suprayogi et al., 2011). A great NHI value indicates high values of grain protein concentration (GPC) (Brevis et al., 2010; Xu et al., 2012); this relationship is supported by Khalilzadeh et al. (2011) who reported that the nitrogen uptake rate and the concentration of nitrogen in grains are effective criteria for the selection of wheat with high NUE. Plants well supplied with nitrogen have higher photosynthetic activity. In addition, Fitzgerald et al., (2010) found a high significant correlation (0.97) between chlorophyll concentration and nitrogen content on the wheat leaves. Furthermore, these plants usually have slow leaf senescence after anthesis, optimizing grain filling and NUE (Van Oosterom et al., 2010; Gaju et al., 2011). Thus, it is possible to estimate leaf nitrogen content and select the best genotypes using portable devices such as chlorophylometers. In breeding programs it is essential to select elite parents based on their ability to transmit desirable traits to offspring. Significant values of specific ability combining (SCA and general combining ability (GCA of

NUE components were found by Le Gouis et al. (2002) in diallel cross, and by Gorny et al. (2011) in $F_{2:3}$ segregating populations of wheat. These results suggest that the extent of genetic variability available on NUE components is sufficient to achieve genetic progress in breeding programs. The occurrence of genetic variability to NUE components in wheat has been reported worldwide: Canada (Krobel et al., 2012), France (Le Gouis et al., 2000), Australia (Hochman et al., 2013), and Argentina (Velasco et al., 2012). However, there are no reports of similar studies with Brazilian wheat genotypes. Therefore, the objective of this study was to investigate the combining ability of NUE components and important agronomic traits in F_2 wheat populations and try to identify new selection criteria to succeed genetic progress in early segregating generations.

Results and Discussion

Analysis of variance and genetic effects

The mean square values from the analysis of variance to general (GCA) and and specific ability combining (SCA) to14 traits are presented in Table 1. The diallel analysis showed significant effects of genotypes for all traits evaluated ($p\leq 0.01$). It indicates that parents and the F2 segregating populations are genetically contrasting and complementary. The values of GCA and SCA were significant ($p\leq0.05$) for all traits, except for the number of spikes per plant (NSP), chlorophyll A index (Chl_A), and chlorophyll B index (Chl_B). This shows that additive and non-additive genetic effects were crucial for the expression of these traits. Coefficients of variation ranged from 1.89 to 12.70% and selective accuracy varied from 0.70 to 0.99, demonstrating a high degree of experimental accuracy and reliability of the statistical inferences tested. Baker's ratio is useful to demonstrate the presence of additive and non-additive effects of genes on the expression of traits. The larger the values (i.e., closer to 1), the greater the contribution of additive genetic effects is in the expression of traits (Baker, 1978). In the present study, these values were near to 1 for most part of the traits evaluated, except NSP and nitrogen harvest index (NHI) (Table 1). These results are consistent with those reported by Gorny et al. (2011). Baker's ratio observed to grain yield (GY) was 0.99, which is higher than values reported by Zare-Kohan and Heidari (2012). On the other hand, the values observed for NSP (0.70) and NHI (0.73) indicate a moderate involvement of non-additive effects playing an important role.

Mean performance and grain yield components

Grain yield ranged from 2652 kg ha^{-1} to 6125 kg ha^{-1}, classifying genotypes into five equal groups according to the Scott and Knott test (Table 2). The group with the highest values of GY included the parents Mirante (6034 kg ha^{-1}) and F. Raízes (5534 kg ha^{-1}), and the combinations Mirante × Valente (5962 kg ha^{-1}), Mirante BRS × Tangará (6125 kg ha^{-1}) and Mirante × F. Raízes (5983 kg ha^{-1}), all differing significantly from the other genotypes ($p\leq0.05$). These same genotypes also presented the greatest values for kernels per spike (KPS) and Chl_B. These results agree with Tian et al. (2011) and Wani et al. (2011), who also reported that more productive genotypes tend to show high values of relative Chl_B content. This is probably due to high leaf nitrogen concentration, as a result of more efficient absorption of this nutrient from soil (Fitzgerald et al., 2010). The yield components of parents and segregating populations were divided into two groups for NSP, three groups for KPS, and four groups for test weight (TW) and thousand kernel weight (TKW). This large variability can be attributed to the genetic dissimilarity of the wheat parents used in the study, which includes parents released in different periods. Among the F_2 segregating population, variations were observed in the following traits: NSP (5.1 to 8.8), KPS (32 to 46), TW (70 to 81) and TKW (28 to 40). These results are similar to those obtained by Fellahi et al. (2013), who reported TW and KPS variations of 8.4 g and 15.6 grains, respectively.

Nitrogen use efficiency and its components

The total biomass nitrogen concentration (BNC) ranged from 2.38 to 3.27% and grain protein concentration (GPC) varied between 10.81 and 15.53 % (Table 2), confirming the results of Bogard et al. (2010), who observed GPC values ranging from 10.16 to 13.98%. Likewise, Cormier et al. (2013) reported genetic progress to a straw nitrogen concentration at physiological maturity of -0.52% $year^{-1}$ due to greater efficiency of nitrogen remobilization in modern cultivars. Thus, genotypes with low BNC and high NHI are essential to improve NUE, because they are more efficiently remobilize the nitrogen from straw to grains (Kichey et al., 2007; Cormier et al., 2013). Therefore, according to the results shown in Table 2, the crosses Mirante × Valente and Mirante × BRS Tangará are promising combinations to be selected as superior lines for high NUE. The parents BRS Tangará and Toropi showed high values of grain nitrogen concentration (GNC) (2.72 and 2.62%) and GPC (15.53 and 14.94%), respectively. According to Hruskova (2009), this demonstrates that these genotypes are more efficient in the absorption of nitrogen available in soil. The crosses Mirante × Valente, BRS Tangará × F. Raízes, and BRS Tangará × Toropi also showed pronounced performance for these traits. GNC and GPC are strictly related to the end-use quality of wheat (Brevis et al., 2010); consequently, they are important attributes to be considered in breeding programs. Nitrogen use efficiency by grains (NUEg) indicates how many kilograms of grain a given genotype produces for each kilogram of nitrogen available, while NHI is calculated by dividing grain nitrogen content by the nitrogen concentration of the total biomass above-ground (straw+grains) (Moll et al., 1982). In this study, NUEg ranged from 45.6 to 82.6 kg kg^{-1}, highlighting the parents Mirante (81.5) and F. Raízes (74.8) and the crosses Mirante × Valente (80.6), Mirante × BRS Tangará (82.7), and Mirante × F Raízes (80.8) (Table 2); these are similar to results reported by Barraclough et al. (2010). Genotypes with high GY tend to present low GNC (Bogard et al., 2010); nonetheless, they also tend to have high NUEg values (Xu et al., 2012). This behavior was observed for the parents Mirante and F. Raízes. NHI values ranged from 69 to 86% (Table 2), similar to results described in other studies (Barraclough et al., 2010; Suprayogi et al., 2011). The parents and crosses with the largest values for NHI were Valente (81.6%), BRS Tangará (83.3%), Toropi (82.6%), Mirante × Valente (83%), Mirante × BRS Tangará (85%), Mirante × F. Raízes (84%), Mirante × CD 115 (84.3%), Valente × BRS Tangará (86.7%), Valente × F. Raízes (82.6%), Valente × Toropi (85.7%), and F. Raízes × Toropi (82.3%). Cormier et al. (2013) reported that the key factor in the progress of NUE is to achieve better partitioning of nitrogen through an increase in NHI followed by a decrease in BNC at physiological maturity. Genotypes with high NHI allow put together high yields and great GPC values (Hawkesford, 2012). The production protein efficiency (PPE) is also an

Table 1. Mean squares of the analysis of variance diallel (parents and F_2) for yield components, chlorophyll content, Kjeldahl traits and NUE components analyzed by the model proposed by Griffing (1956) in spring wheat.

Traits	Variation sources and mean squares										
	Blocks *(DF=2)*	Genotypes *(DF=20)*	GCA *(DF=5)*	SCA *(DF=5)*	Error *(DF=40)*	SA	CV(%)	σ^2_g	σ^2_E	H^2	Baker's ratio
Yield components											
NSP	0.62 ^{ns}	3.06**	3.36^{ns}	2.97**	0.77	0.86	12.7	0.76	0.77	0.75	0.70
KPS	30.12**	51.59**	119.76*	28.86*	12.93	0.87	8.82	12.89	12.93	0.75	0.93
TW (kg hl^{-1})	9.30**	30.64**	96.42**	8.72**	2.12	0.96	1.89	9.51	2.12	0.93	0.97
TKW	0.96 ^{ns}	32.01**	105.01**	7.78**	2.12	0.97	4.26	9.99	2.12	0.93	0.97
GY(kg ha^{-1})	27973 **	2694852**	10184971**	198142**	78030	0.99	5.83	87227	78030	0.97	0.99
Relative chlorophyll content (Falker index)											
Chl_A	37.91**	2.51*	4.93*	1.68^{ns}	1.29	0.70	3.22	0.40	1.29	0.48	0.95
Chl_B	1.84 ^{ns}	1.30**	3.78**	0.46^{ns}	0.27	0.90	4.19	0.34	0.27	0.79	0.97
Chl_{A+B}	34.53**	5.05**	10.33*	3.35*	1.56	0.83	2.62	1.16	1.56	0.69	0.91
Analytical determinations by Kjeldahl method											
$GNC_{(\%)}$	0.04 ^{ns}	0.16**	0.34**	0.11*	0.02	0.93	6.54	0.05	0.02	0.87	0.88
$BNC_{(\%)}$	0.06 ^{ns}	0.18**	0.32**	0.13*	0.03	0.92	5.83	0.05	0.03	0.85	0.85
$GPC_{(\%)}$	1.20 ^{ns}	5.55**	11.36**	3.61**	0.73	0.93	6.54	1.61	0.73	0.87	0.88
NUE components											
NUEg(kg	5.10*	492.08**	1859.75*	36.19*	14.25	0.99	5.83	159.30	14.25	0.97	0.99
NHI	26.98**	67.45*	82.88*	62.31**	7.01	0.94	3.50	20.14	7.01	0.89	0.73
PPE(kg kg$^-$	0.18 ^{ns}	8.76**	27.88*	2.39*	0.60	0.97	9.15	2.73	0.60	0.93	0.97

** and * are values significant at 1% ($p \leq 0.01$) and 5% ($0.01p \leq 0.05$) level of probability by F test; ^{ns}: not significant ($p \leq 0.05$); DF: degrees of freedom; SA: selective accuracy; CV: coefficient of variation; H^2: broad sense heritability; GCA: general combining ability , SCA: specific combining ability; NSP: number of spikes per plant, KPS: kernels per spike; TW: test weight; TKW: thousand kernel weight; GY: grain yield; Chl_A: Chlorophyll A index; Chl_B: Chlorophyll B index; Chl_{A+B}: Total chlorophyll content index; GNC: grain nitrogen concentration; BNC: biomass nitrogen concentration; GPC: grain protein concentration; NUEg: nitrogen use efficiency by grains; NHI: nitrogen harvest index; PPE: production protein efficiency.

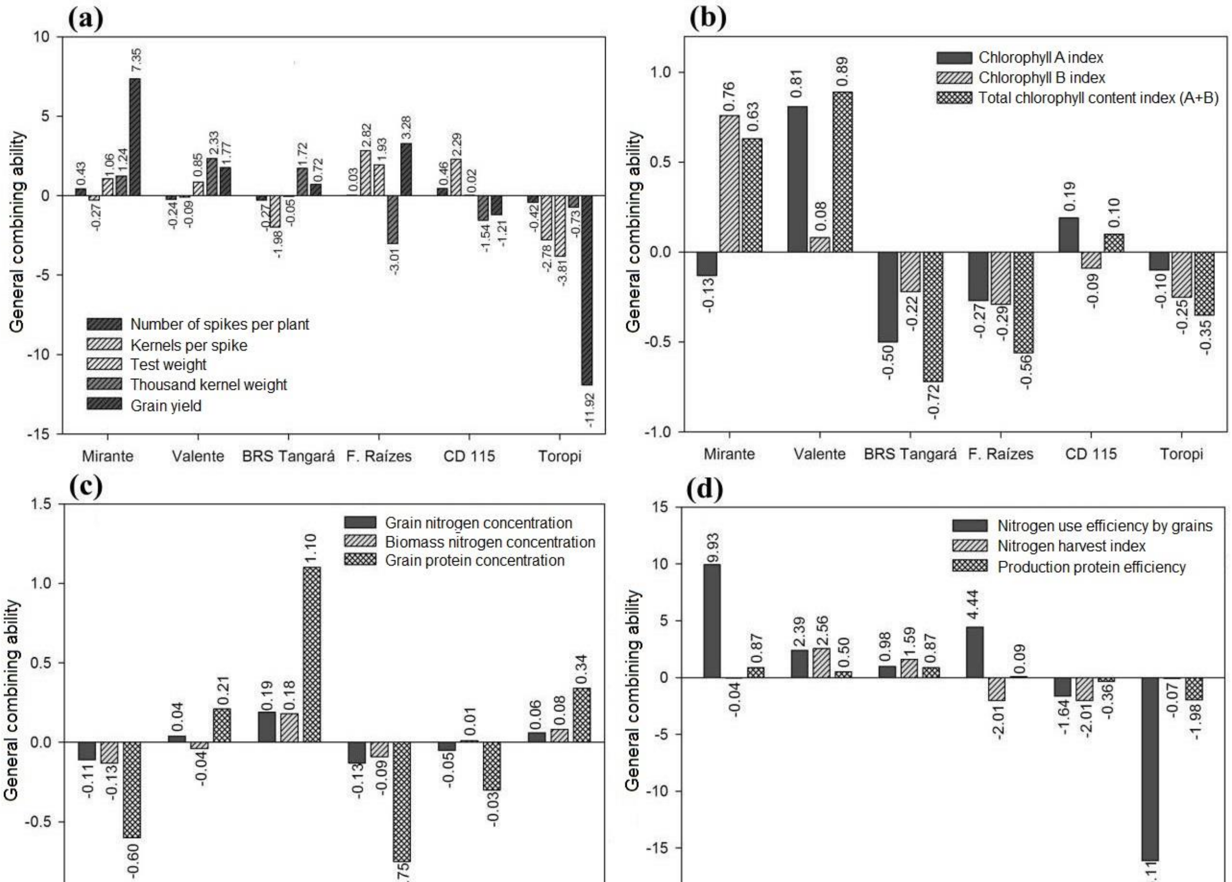

Fig 1. Values of general combining ability for yield components (a), chlorophyll content (b), N traits determined by the Kjeldahl method (c) and NUE components (d) evaluated in a wheat diallel (parents and F_2) according to the model 2 proposed by Griffing (1956).

Table 2. Mean performance of six wheat parents and their respective hybrids derived from a diallel cross for a set of 14 traits, including yield components, chlorophyll content, N traits determined by the Kjeldahl method and NUE components.

Genotypes	Yield components					Chlorophyll content			Kjeldahl's analyses			NUE components		
	NSP	KPS	TW kg hl^{-1}	TKW grams	GY kg ha^{-1}	Chl $_A$	Chl $_B$	Chl $_{A+B}$	GNC %	BNC %	GPC %	NUEg kg kg^{-}	NHI %	PPE kg kg^{-1}
Parents														
Mirante (1)	6.6 b	38 b	77 b	35 b	6034 a	34 b	13 a	47 b	1.8 c	2.5 d	10.8c	81 a	73 c	8.8 b
Valente (2)	6.8 a	39 b	79 a	40 a	5280 b	36 a	12 b	48 a	2.1 c	2.6 d	12.2 c	71 b	81 a	8.7 b
BRS Tangará (3)	6.3 b	33 c	77 b	37 b	4912 c	34 b	12 b	46 b	2.7 a	3.2 a	15.5 a	66 c	83 a	10.3 a
F. Raízes (4)	7.0 a	43 a	81 a	28 d	5534 a	34 b	11 b	46 b	1.9 c	2.8 c	11.2 c	74 a	69 c	8.4 b
CD 115 (5)	7.4 a	46 a	80 a	31 c	4681 c	36 a	12 b	48 a	2.0 c	2.6 d	11.4 c	63 c	75 b	7.2 c
Toropi (6)	5.9 b	32 c	71 d	30 d	2652 e	34 b	11 b	45 b	2.6 a	3.1 a	14.9 a	35 e	82 a	5.3 d
Mean	*6.7*	*39*	*77*	*33*	*4848*	*34*	*11*	*46*	*2.2*	*2.8*	*12.7*	*65*	*77*	*8.1*
F_2 population														
1 × 2	7.8 a	45 a	79 a	37 b	5962 a	37 a	13 a	51 a	2.5 a	3.0 b	14.3 a	80 a	83 a	11.5 a
1 × 3	8.8 a	40 b	78 b	36 b	6125 a	34 b	12 a	47 b	2.3 b	2.7 c	13.1 b	82 a	85 a	10.9 a
1 × 4	8.5 a	39 b	80 a	33 c	5983 a	34 b	13 a	48 b	2.0 c	2.3 d	11.4 c	80 a	84 a	9.2 b
1 × 5	7.4 a	41 a	79 a	34 b	5269 b	34 b	13 a	47 b	2.5 a	3.0 b	14.5 a	71 b	84 a	10.3 a
1 × 6	5.7 b	39 b	74 c	35 b	3989 d	36 a	13 a	49 a	2.0 c	2.7 c	11.8 c	53 d	75 b	6.3 d
2 × 3	5.1 b	40 b	77 b	37 b	4745 c	35 a	12 b	47 b	2.5 a	2.9 b	14.5 a	64 c	86 a	9.3 b
2 × 4	5.5 b	46 a	79 a	33 c	4926 c	35 b	12 b	47 b	2.3 b	2.8 c	13.3 b	66 c	82 a	8.9 b
2 × 5	7.0 a	39 b	75 c	33 c	4897 c	36 a	12 a	48 a	2.2 b	2.9 b	12.9 b	66 c	77 b	8.5 b
2 × 6	7.1 a	34 c	75 c	36 b	3842 d	35 a	12 a	48 a	2.4 b	2.8 c	13.7 b	51 d	85 a	7.1 c
3 × 4	6.4 b	45 a	80 a	32 c	5232 b	34 b	11 b	45 b	2.4 a	3.1 a	14.1 a	70 b	78 b	10.0 a
3 × 5	7.9 a	41 a	75 c	33 c	4502 c	34 b	11 b	46 b	2.3 b	2.9 b	13.3 b	60 c	79 b	8.1 b
3 × 6	5.2 b	36 c	73 c	39 a	3667 d	34 b	12 b	46 b	2.4 a	3.2 a	14.1 a	49 d	75 b	7.0 c
4 × 5	6.8 a	44 a	78 b	29 d	5139 b	35 b	11 b	46 b	2.2 c	2.8 b	12.5 c	69 b	76 b	8.7 b
4 × 6	7.1 a	45 a	72 d	28 d	3795 d	36 a	11 b	48 b	2.0 c	2.5 d	11.8 c	51 d	82 a	6.0 d
5 × 6	7.8 a	42 a	70 d	33 c	3377 d	35 b	12 b	47 b	2.2 b	3.0 b	12.9 b	45 d	73 c	5.9 d
Mean	*6.9*	*41*	*76*	*33*	*4763*	*34*	*12*	*47*	*2.3*	*2.8*	*13.2*	*63*	*80*	*8.5*

NSP: number of spikes per plant, KPS: kernels per spike; TW: test weight; TKW: thousand kernel weight; GY: grain yield; Chl_A: Chlorophyll A index; Chl_B: Chlorophyll B index; Chl_{A+B}: Total chlorophyll content index; GNC: grain nitrogen concentration; BNC: biomass nitrogen concentration; GPC: grain protein concentration; NUEg: nitrogen use efficiency by grains; NHI: nitrogen harvest index; PPE: production protein efficiency. * Means followed by the same letter do not differ between them by the Scott and Knott test at 5% probability of error.

Table 3. Estimates of specific combining ability (GCA) for yield components, chlorophyll content , N traits determined by the Kjeldahl method and NUE components evaluated in a wheat diallel (parents and F_2) according to the model 2 proposed by Griffing (1956).

Crosses	1 × 2	1 × 3	1 × 4	1 × 5	1 × 6	2 × 3	2 × 4	2 × 5	2 × 6	3 × 4	3 × 5	3 × 6	4 × 5	4 × 6	5 × 6
Yield components															
NSP	0,79	1,71	1,20	-0,32	-1,20	-1,27	-1,13	-0,12	0,8	-0,2	0,8	-0,9	-0,5	0,6	0,9
KPS	4,59	1,49	-3,45	-1,26	1,35	1,51	2,50	-3,09	-3,7	3,4	0,5	0,1	-1,3	4,8	2,2
TW	0,39	0,46	0,15	1,39	-0,05	-0,40	-0,08	-2,18	1,0	1,2	-1,9	0,2	-0,4	-2,5	-2,8
TKW	-0,22	-0,52	1,52	0,80	1,09	-1,10	0,37	-1,54	0,2	-0,2	-0,8	3,9	-0,6	-1,6	1,5
GY	2,62	5,29	1,31	-1,33	-3,41	-2,92	-3,67	0,53	0,7	0,4	-2,4	-0,0	1,4	-1,3	-0,9
Relative chlorophyll content (Falker index)															
Chl $_A$	1,57	-0,28	0,00	-0,67	0,97	0,00	-0,74	-0,13	-0,1	-0,4	-0,1	0,1	-0,2	1,2	-0,4
Chl $_B$	0,37	-0,02	0,26	-0,04	0,62	-0,06	-0,08	0,32	0,4	-0,4	-0,4	0,1	-0,1	0,1	0,2
Chl $_{A+B}$	1,96	-0,23	0,21	-0,76	1,59	0,01	-0,86	0,18	0,3	-0,7	-0,5	0,2	-0,2	1,3	-0,2
Analytical determinations by Kjeldahl method															
GNC	0,29	-0,08	-0,06	0,41	-0,18	0,02	0,14	0,00	0,0	0,1	-0,1	-0,1	0,1	-0,2	-0,0
BNC	0,33	-0,21	-0,29	0,28	-0,07	-0,07	0,08	0,09	-0,1	0,2	-0,1	0,1	0,1	-0,4	0,1
GPC	1,64	-0,42	-0,35	2,35	-1,04	0,14	0,80	-0,03	0,1	0,7	-0,5	-0,4	0,5	-0,9	-0,2
NUE components															
	3,53	7,16	1,77	-1,80	-4,61	-3,95	-4,96	0,71	0,9	0,6	-3,2	-0,0	1,9	-1,7	-1,3
	0,69	3,53	6,39	6,50	-4,59	2,60	2,40	-2,45	3,1	-1,0	-0,2	-5,4	0,6	4,5	-3,8
	1,77	0,73	-0,17	1,39	-0,97	-0,48	-0,09	-0,01	0,2	0,6	-0,8	-0,3	0,6	-0,5	-0,2

NSP: number of spikes per plant, KPS: kernels per spike; TW: test weight; TKW: thousand kernel weight; GY: grain yield; Chl_A: Chlorophyll A index; Chl_B: Chlorophyll B index; Chl_{A+B}: Total chlorophyll content index; GNC: grain nitrogen concentration; BNC: biomass nitrogen concentration; GPC: grain protein concentration; NUEg: nitrogen use efficiency by grains; NHI: nitrogen harvest index; PPE: production protein efficiency.

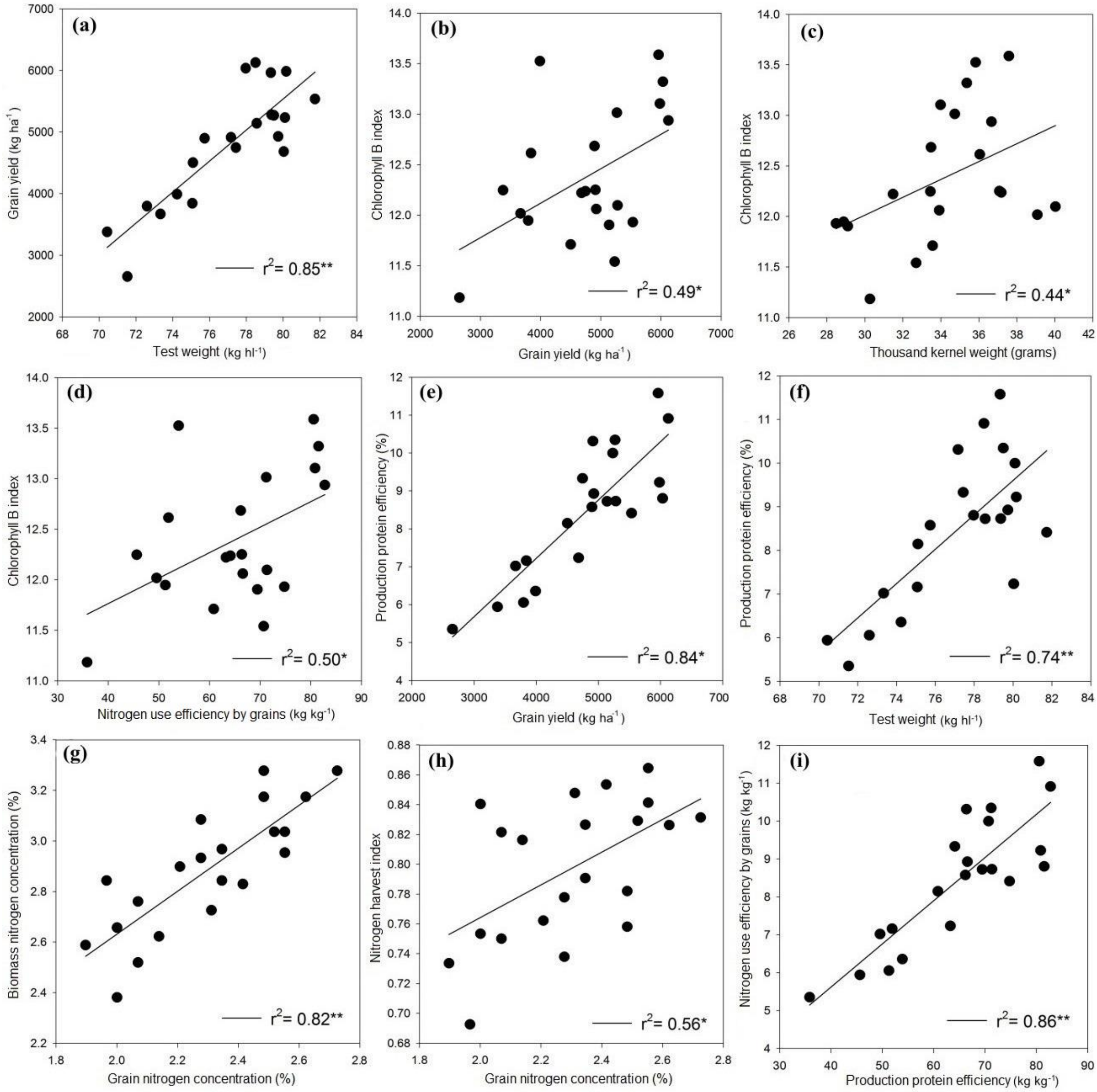

Fig 2. Pearson's correlation coefficients between agronomic traits and NUE components in a diallel cross of wheat. * and** indicate significant values of correlation to 1 and 5% level of probability, respectively, by t test (GL-2).

important NUE component, because it specifies how many kilograms of protein are produced (kg ha^{-1}) for each kilogram of nitrogen available in the soil (Haile et al., 2012). The parent BRS Tangará (10.3) as well as the crosses Mirante x Valente (11.5), Mirante × BRS Tangará (10.9), Mirante × CD 115 (10.3), and BRS Tangará × F. Raízes (10.0) stood out with high PPE indexes in this study.

Estimates of General and specific ability combining

General combining ability (GCA) indicates the contribution of additive genetic effects that can be fixed in segregating populations. In this study, the parents Mirante and F. Raízes showed the highest positive values of GCA to GY (7.35 and 3.28) and KPS (1.06 and 2.82), respectively (Figure 1a), qualifying them as good combiners that should be prioritized into crossing blocks. Valente stood out as the best combiner to generate segregating populations with high TKW (GCA= 2.33). In contrast, Toropi decreased the GY and its components in the crosses that was it was present. This cultivar was released in 1965; as a result it added little positive genetic effects to the traits studied. The parents Mirante and F. Raízes showed high values of GCA for Chl_B (0.76) and Chl_A traits (0.81), respectively; they also were good combiners due to increasing total chlorophyll content (Chl_{A+B}) (Figure 1b). Several studies have been done indicating that chlorophyll content is positively associated with GY and its components (Tian et al., 2011; Wani et al., 2011), which agrees with our results where the parents Mirante and Valente stood out with great GCA values simultaneously to both of these traits. Parents BRS Tangará and Toropi exhibited the highest values of GCA to GNC (0.19 and 0.06), BNC (0.18 and 0.08), and GPC (1.10 and 0.34) respectively, (Figure 1c). Although Toropi provided improvements in the traits mentioned above, the reduction in the expression of yield components makes it difficult to use

Toropi directly for improving the traits studied; however, it still might be useful to the development of germplasm. The low values of GCA estimates to GNC refer to the difficulty of obtaining breeding progress for this trait, according to results obtained by Cormier et al. (2013). The highest values of GCA for NUEg were expressed by the parents Mirante and F. Raízes (9.93 and 4.44, respectively). Valente (2.56) and BRS Tangará (1.59) presented the greatest values of GCA to NHI (Figure 1d). Additionally, Mirante and BRS Tangará stood out for GCA (0.87) of the trait PPE. These results corroborated with Gorny et al. (2011) and Yildirim et al. (2007), which reported a similar magnitude of values and found that additive genetic effects (related to CGC) had greater importance to NUE components in F_2 segregating populations of wheat. The specific combining ability (SCA) is related to dominance and non-additive effects, which point out how hybrids can be superior or inferior to their parents (Izge and Garba, 2012). The SCA has no direct contribution to the breeding of pollinated plants, except as a feasible explanation of heterosis. However, hybrid populations with higher SCA values generate a higher frequency of transgressive segregants and increase the probability of selecting superior inbreeding lines (Zare-Kohan and Heidari, 2012; Fellahi et al., 2013). In general, all traits studied showed low values of SCA (Table 3), indicating that the behavior of this population was well-predicted by the GCA estimates. Nevertheless, the SCA values of some crosses stand out; for example, Mirante × Valente (2.62) and Mirante × BRS Tangará (5.29) showed high SCA estimates for GY. Mirante × Valente also presented high SCA values to KPS (4.59) and Chl_{A+B} (1.96), while Mirante × BRS Tangará excelled with high SCA to NSP (1.71), indicating a high degree of allelic complementarity among these wheat parents. The combination Mirante CD × 115 presented a high magnitude of SCA to the following traits: GNC (0.41), BNC (0.28), GPC (2.35), NHI (6.5), and PPE (1.39) (Table 3). Furthermore, the combinations Mirante × Valente and Mirante × BRS Tangará also stood out, with high SCA values to NUEg (3.53 and 7.16) and PPE (1.77 and 0.73), respectively. It shows that these parents are efficient in their use of nitrogen to fill grains and increase protein content. These results are in agreement with Gorny et al. (2011), who also reported similar SCA values, ranging from -4.48 to 3.80 for EUNg in Poland.

Grain yield and NUE components are associated with chlorophyll content

Positive correlations of moderate to high magnitude indicate that genotypes with a higher relative chlorophyll index B (Chl_B) tend to show high GY (0.49*) and TW (0.44*) (Figures 2b and 2c). Tian et al. (2011) and Wani et al. (2011) also verified a moderate association between Chl_B and GY and its components in wheat. The trait Chl_B was positively associated with NUEg (0.50*) (Figure 2d), showing that the genotypes with the highest concentration of photosynthetic pigment tend to be more efficient in the use of nitrogen for grain filling. These results agree with those obtained by Fitzgerald et al. (2010), who found a correlation of 0.97 between chlorophyll concentration and nitrogen leaf content.
BNC was highly associated with GNC (0.82**) (Figure 2g), which in turn is correlated with NHI (0.56*) (Figure 2h). These associations are explained by the remobilization of nitrogen accumulated from the biomass to the grain after anthesis (Suprayogi et al., 2011). Additionally, a vastly significant correlation was found between NUEg and PPE (0.86**), demonstrating that genotypes with more efficient use of nitrogen to produce grains also are more efficient in enhancing protein content. The parental choice should focus on genotypes with higher GCA values (Izge and Garba, 2012), which increases the likelihood of recovering superior inbred lines. Among the parents tested, Mirante, BRS Valente, and Tangará tend to bring together more characteristics of interest, indicating that they are promising sources of genes to be exploited in breeding programs. Also noteworthy is the feasibility of using chlorophyll B analyses for selection targeting to increase GY and NUE in segregating populations. These results provide consistent information that can be used to adopt new selection criteria for selecting superior lines in wheat breeding programs.

Material and Methods

Experimental design and crop management

Six wheat parents (Mirante, Fundacep Raízes, CD 115, Toropi, BRS Tangará, and Valente) were hybridized in a complete diallel without reciprocals, totaling fifteen segregating populations. In 2010, the F_1 seeds were sown in a greenhouse in order to obtain F_2 seeds. The experiment was sown in an area managed under no-tillage on July 16, 2011 in Pato Branco, Paraná State, Brazil (26°10' S and 52º41'W). Soil testing of the area before the experiment showed: pH 5.6 ($CaCl_2$); 0.00 Al (cmol dm^{-3}) 0.43 K (cmol dm^{-3}); 13.75 P (mg dm^{-3}); 56.29 OM (g dm^{-3}); and 70.36 % of base saturation. The fifteen F_2 segregating populations and six parents were planted in the field in a randomized block design with three replications. Plots contained nine rows, 3.0 m in length, spaced 0.20 m apart (5.4 m^2), with a seeding density of 150 plants per m^2. Base fertilization consisted of applying 24 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1} of commercial formulation 8-20-20 (NPK). At the beginning of tillering (Z22 stage, Zadocks et al., 1974), the fertilization was complemented with 50 kg ha^{-1} of N in urea form (45% N). The control of weeds, pests and diseases were performed according to the technical recommendations for wheat (Rcbtt, 2010).

Measurements of agronomic traits

The number of spikes per plant (NSP) and kernels per spike (KPS) were determined by sampling 20 plants randomly from each plot. The test weight (TW) was assessed using the determined grain moisture (G800, Gehaka model), and thousand kernel weight (TKW) was obtained by counting the four samples, each with fifty grains, and expressing the results in grams. Grain yield (GY) was measured by harvesting the whole area of each plot, then corrected to 13% moisture (wet basis) and converted to kg ha^{-1}. The individual and total chlorophyll contents (Chl_A, Chl_B, Chl_{A+B}) were measured in flag leaves during the post-anthesis stage (Z65 stage; Zadoks, 1974). The data were collected from healthy, fully expanded leaves from ten plants per plot using a portable electronic chlorophyll meter (model ClorofiLOG CFL 1030).

Nitrogen use efficiency analysis

At physiological maturity (Z90 stage, Zadocks et al., 1974), ten plants per plot were collected to determine the nitrogen concentration in the straw (SNC) and grains (GNC). After that, the samples were dried at 40ºC to a constant weight and triturated in an analytical mill (IKA A11, model BS32). Subsequently, the extracts were subjected to chemical analysis to determine the nitrogen concentration using the Kjeldahl method, according to Tedesco et al. (1995). Grain protein concentration (GPC) was estimated by multiplying the grain nitrogen concentration (GNC) by a factor of 5.7 (AACC, 2000). NUE components were estimated according to Moll et al. (1982) and Weih et al. (2011) and include the following traits:

- ***Nitrogen use efficiency by grains (NUEg)*:**

$NUE_{g\,(kg\,kg^{-1})} = GY/NS$

Where: GY= grain yield (kg ha^{-1}), and NS= nitrogen supply (kg ha^{-1}).

- ***Production protein efficiency (PPE)***: $PPE_{\,(kg\,kg^{-1})} = ((GPC\ \times GY)/100)/NS$

Where: GPC= grain protein concentration (%), GY=grain yield (kg ha^{-1}), and NS= nitrogen supply (kg ha^{-1}).

- ***Nitrogen harvest index (NHI)***:

$NHI_{(\%)} = GPC\ \times GY/((GNC + ENC) \times BIO)$

Where: GPC= grain protein concentration, GNC= grain nitrogen concentration, ENC= straw nitrogen concentration, GY= grain yield (kg ha^{-1}), and BIO= total biomass above ground (straw+grains) in kg ha^{-1}.

Data analysis

Data were subjected to statistical analysis of variance, considering fixed effects to genotypes. The sums of squares of treatments were then divided into general (GCA) and specific combining ability (SCA), through diallel analysis of Griffing (method 2). All traits that were found significant by F test were grouped using the Scott and Knott test (1974), at 5% level of significance ($p \leq 0.05$).

Additionally, we estimated the Pearson's correlation among all traits evaluated. The broad-sense heritability was calculated by dividing the genotypic variance by the phenotypic variance: $H^2 = \sigma^2_G / \sigma^2_P$. The relative importance of GCA in the determination of the performance of each hybrid combination was measured according to Baker (1978) with the expression $2\sigma^2{}_{GCA}/(2\sigma^2{}_{GCA} + \sigma^2{}_{SCA})$. All statistical analyses and graphs were attained using the Genes program (Cruz, 2013) and the Sigmaplot software.

Conclusions

Genetic variability and additive and non-additive gene effects influence the components of nitrogen use efficiency in segregating populations of wheat. The parents Mirante, BRS Tangará, and Valente are promising sources of favorable genes for NUE components. The relative chlorophyll B index can be used as a selection criterion to identify superior crosses for grain yield and nitrogen use efficiency in early segregating generations.

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