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RESEARCH ARTICLE

Stay green behaviour of a novel mutant, PSG16 shows complex inheritance and functional relations with grain yield in rice

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Abstract

To ensure food security for the future, contemporary rice breeding has to harness novel secondary traits linked to higher productivity. Functional staygreen (SG) is one such trait that has shown promise by delaying senescence while improving crop productivity. Pusa-stay-green 16 (PSG 16) is a novel SG mutant that appeared in a breeding population that showed true to type breeding in subsequent generations and higher grain yield. Study of the inheritance pattern of the SG behavior in PSG16, by crossing with two non-SG parents Pusa 677 and Pusa 44 indicated a major gene inheritance in the F_2 generation. The trait was found associated with the measure of total chlorophyll content (TCC) at the physiological maturity stage. The relationship of TCC to other agronomic traits, in the segregating populations of the cross, from F_2-F_5, showed a consistent association with grain yield, suggesting the functional property of the SG trait. However, TCC showed a quantitative inheritance pattern. Additional investigations on the inheritance pattern and gene actions for the TCC revealed significant additive and dominant gene actions. The dominance superseded the additive component on an individual gene basis, thereby signifying the class variation observed under the goodness of fit test. However, a large interaction component with additive x additive gene action could be identified in trait expression. Therefore, the inheritance of SG trait was complex and deviated from the simple additive dominance pattern. A careful breeding procedure involving intermating, sib-mating and recurrent selection may be needed to utilize the trait in crop improvement. The causative loci need to be mapped for further understanding of the SG behavior of PSG16, along with in-depth physiological characterization for the functionality of the trait.

Keywords: Epistasis, Gene action, Grain yield, Inheritance, Stay green.

Introduction

Rice (*Oryza sativa* L.) dominates as one of the most important food crops providing dietary supplies to nearly 60% of the world population (Dai G. J. et al. 2015). Increasing crop yield of major cereal crops like rice and wheat has been breeders' most important but challenging goal. Global human population growth and climate change are two prominent contemporary challenges, which call for accelerated rice breeding. To sustain future food grain production, discovering novel secondary traits related to higher productivity and stress resilience has become a mandatory component of breeding goals (Abdelrahman et al. 2017). The interaction of two major nutrient elements, carbon (C) and nitrogen (N) plays a prime role in defining crop productivity. In rice, 60–90% of the total grain C content during physiological maturity is assimilated through photosynthesis after flowering, while 80% of N is channelized from the reservoir accrued before flowering (Mae 1997). The N pool is initially built in the vegetative parts of the rice plant and subsequently translocated to grains. These relations signify the importance of the stay-green (SG) trait, in which the leaf senescence is delayed during grain development and maturation so that photosynthetic support to the developing grains remains extended. The utilization of the SG trait to enhance rice yield as an alternative strategy was first proposed by Khush (2000). From the earlier experience in other crops, SG was found to delay ribulose-1,5-bisphosphate carboxylase/oxygenase

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(Rubisco) degradation, prolonging canopy photosynthesis and higher yield in some genotypes (Evans 1993). SG occurs in two forms, functional and non-functional, and is a physiological modification of senescence machinery in plants. The prolonged photosynthetic efficiency to support crop production happens only in functional SG, whereas non-functional ones have only cosmetic value. Hence, the functional SG trait is considered as an important physiological trait that conditions plants to remain green and photosynthetically active aiding improved the grain-filling even under stress conditions (Zhang et al. 2019). Therefore, breeding functional SG trait is important for achieving improved yield, especially when combined with other yield contributing traits.

Recognizing the importance of SG trait, several genetic investigations were carried out in rice. Fu et al. (2011) mapped two SG-linked QTLs, such as *Csfl6* and *Tcs9* sharing the same genomic locations with grain yield associated QTLs, *Yld6* and *Yld9*. The colocalization of QTLs establishes connectivity between the SG trait and high crop productivity in rice. Jiang et al. (2004) extensively analyzed the QTLs for delayed leaf senescence using a doubled haploid population from an *indica-japonica* cross, Zhenshan 97/Wuyujing 2, and reported a total of 46 QTLs in 25 chromosomal locations. They confirmed that the *japonica* parent was found to contribute extensively to delayed senescence at all but one QTL. The differential senescence identified in the *indica-japonica* cross combination was not because of overdominance, but due to partial dominance of genes contributed by either of the parents. Most of the QTLs for senescence were mapped on the short arm of linkage group 6 and the long arm of linkage group 9 of rice (Abdelkhalik et al. 2005). Associated with delayed senescence, several genomic regions for physiological and agronomic traits such as Rubisco activity, chlorophyll content and ratooning ability were identified by Ishimaru et al. (2001), using a backcross inbred lines population generated from a *japonica* × *indica* cross involving Nipponbare and Kasalath. Several non-allelic interactions among and between SG loci in rice have been identified (Yang et al. 2003).

Nevertheless, the genetics of SG trait in rice remains obscure due to lack of intense investigations. Unlike in crops like sorghum and wheat, SG trait in rice is relatively low and functional SG variance are rare. Once identified, recombinant inbred lines (RILs) can be generated from an SG variant to map the causative loci, unfold complex epistatic interactions, and identify phenotypes associated with the SG trait and grain yield. However, quantifying SG trait has proved to be cumbersome, especially in segregating generations where a wide range of trait manifestation occurs. Delayed senescence in rice depends on the subspecies - *indica* senesce faster than *japonica*, and the environment - senescence at a warmer region is quicker than in a cooler region (Yoshida 1981), along with variation in other traits such as heading time, that can expose individual plants to different environmental conditions during the terminal stage. In this study, we utilized a novel spontaneous functional SG mutant, Pusa Stay Green 16 (PSG16) characterized by its robust growth, high tillering ability and grain yield, to study the genetics of SG trait, and relation to grain yield. This would pave way for a newer route for improving crop productivity in rice, particularly under different nutrient input systems, since N is a key player in the expression of delayed senescence.

Materials and methods

A novel stay green (SG) mutant, PSG16 appeared in a breeding population during the late *rabi* season of 2015-16 in the experimental fields of Indian Agricultural Research Institute at the Rice Breeding and Genetics Research Centre (IARI-RBGRC), Aduthurai in Tamil Nadu. The mutant was robust with several tillers and showed prominent SG trait with dark green leaves, even after the grains have matured. The mutant was high-yielding and had bolder grains. Further, advancement of the SG line showed it is true-breeding, with a duration of about 100-105 days. Crosses were made between PSG16 and Pusa 677 and also with Pusa 44, short and long-duration cultivars, respectively. PSG16 was used as the male parent. Pusa 677 is an early flowering and non-stay-green (NSG) *indica* cultivar with 90 days duration, with a rapid senescence pattern, while Pusa 44 is a popular NSG cultivar with a longer duration of 140 days. The cross, Pusa 677/PSG16 was further selected to develop a RIL population for further studies. The generations up to F_5 were developed in subsequent seasons.

Raising the generations

Following the hybridization made during 2016, for the two crosses (Pusa 677/PSG16 and Pusa 44/PSG16), parents along with F_1's were field raised during *rabi* 2016 at IARI-RBGRC, Aduthurai. The F_1s were identified based on the morphology which was significantly different from that of the parents, Pusa 677, Pusa 44 and PSG16. The selected F_1s were selfed and only one plant was selected to raise the F_2 population in the subsequent season. During *kharif* 2017, the F_2 generation was raised unreplicated, along with parents. Ten rows of F_2 progenies were flanked by three rows of parents on both sides. Fresh crosses were also made to generate more F_1 plants. A part of the F_2 seeds was stored in cold storage at 4°C. At physiological maturity, a visual count on SG segregants was made from 320 F_2 individuals. For visual assessment, a 1-5 scoring scale was used (Table 1), where scores 1 to 2 indicated SG types (genotypes with delayed leaf senescence) and 3 to 5 score indicated NSG types (genotypes with early leaf senescence/ yellowing type). All the 320 individual F_2 plants scored earlier were harvested individually at physiological maturity, and the seeds were stored for raising F_3 generation.

Table 1 Inheritance of stay greenness of PSG16 in F_2 generation of two crosses

	No. progenies based on scoring					Total	Expected ratio	χ^2 value	Prob.
Cross	5	4	3	2	1				
Pusa 677/PSG16	48	119	71	26	56	320	3:1	0.07	0.80
	238			82					
Pusa 44/PSG16	104	65	69	39	22	299	3:1	1.08	0.30
	238			61					

Scores: 1-2 is stay-green class and 3-5 is non-stay-green class

Among the crosses, Pusa 44/PSG16 was discontinued due to high variation in the maturity time among the progenies. Since Pusa 677 was closer to PSG16 in duration, the progenies matured almost at the same time in the cross Pusa 677/ PSG16, and hence, were used for generation advancement. In the next season, *rabi* 2017, all the five generations were raised together, with P_1, P_2 and F_1 having planted using a randomized complete block design (RCBD) with two replications. F_2 and F_3 families were grown unreplicated. The SG segregants were further characterised based on the total chlorophyll content (TCC), at the physiological maturity stage. TCC was recorded from ten plants in P_1, P_2, and F_1 generations, all the 300 individual F_2 and 320 F_3 families. In F_3, plants were selected based on the SG behaviour of the F_2 plant from which the F_3 family was derived. TCC was recorded non-destructively using a handheld chlorophyll meter (at LEAF® CHL PLUS, FT Green LLC, USA) and expressed in terms of µg.cm^{-2}. For each plant, the chlorophyll measurement was carried out on the basal portion of the leaf just below the flag leaf from five early tillers in each plant. The F_4 and F_5 generations were derived subsequently from the F_3 families by the single seed descent method. These generations were raised in an RCBD replicated twice during *kharif* 2018 at ICAR-IARI, New Delhi and *rabi* 2018 at IARI-RBGRC,Aduthurai, respectively. The experiment was managed using recommended agronomic practices.

Tests of significance

A test of significance for the segregation of SG trait among the F_2 generation progenies was done by Pearson's chi-square (χ^2) test for goodness of fit (Pearson 1900). The χ^2-analysis which tests the significance of deviation of the observed segregation data from expected segregation data was carried out using the following formula,

$$\chi^2 = \frac{\sum_{i=1}^{n} (O_i - E_i)^2}{E_i}$$

where, O_i is the observed frequency and E_i is the expected frequency data of the *i*th class, and *n* is the total number of classes.

To understand the qualitative inheritance of SG trait, a scaling test was performed for TCC (Mather K. and Jinks 1971). Two scales, C and D were used to test the significance of a simple additive-dominance effect. A significant deviation of any one of these scales from zero indicated the absence of a simple additive-dominance system, indicating the presence of non-allelic interactions. Subsequently, a generation mean analysis using the five-generation model involving P_1, P_2, F_1, F_2 and F_3 generations was carried out (Mather 1949; Hayman 1958; Jinks and Jones 1958). Using the model, population parameters such as mean (*m*), additive (*d*), dominance (*h*) and genetic interactions *viz*., additive × additive (*i*), dominance × dominance (*l*) were worked out. The minimum number of effective factors (n_E) differentiating the parents was calculated based Castle-Wright (Castle 1923; Cockerham 1986; Lynch and Walsh 1998), and Burton-Wright formulae (Burton 1951). A potence ratio (PR) indicating the degree of dominance of the trait was also estimated (Smith1952; Peter and Frey 1966).

Variability in agro-morphologic traits

Agronomic data was also recorded at the physiological maturity stage from all the generations. Plants were harvested and agro-morphologic traits such as plant height (cm), panicle length (cm), number of productive tillers per plant, single plant yield (g), grains per panicle, and spikelet fertility were measured. Five plants were measured for each genotype/family, except for the F_2 generation. The agro-morphologic data for each generation were subjected to analysis of variance and correlations were worked out. To determine whether the TCC consistently represented the segregating classes, SG and NSG over different generations, a one-way analysis of variance (ANOVA) was performed across generations (Snedecor and Cochran 1989). Trans-generational relation between the TCC and grain yield was also worked out by correlation analysis. All the computations were carried out using the Data analysis toolpack in Microsoft Excel.

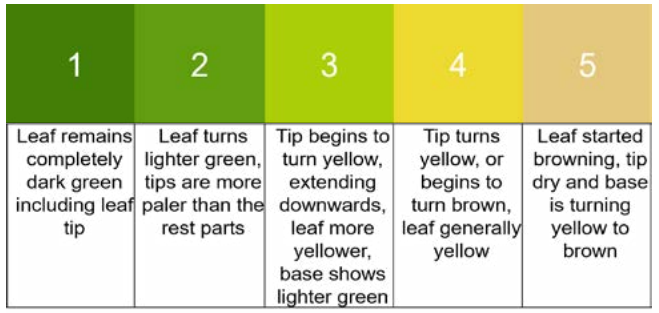
Results and discussion

Because of the extended maintenance of photosynthetic machinery, SG trait in cultivars can leverage improved yield through sustained photosynthesis. Delayed senescence in plants is regulated by internal and external factors, including the sustenance of Rubisco and internal phytohormone balance. Senescence is accelerated by the presence of ethylene and abscisic acid, while cytokinins antagonize the senescence process. Therefore, the physiology of SG is complicated and is under the regulatory fabric of several genes. However, when a mutation occurs to bring in a stay-green feature, the involvement is confined to a single locus that may be either directly or indirectly involved. Therefore, the genetics study in such cases, should begin with an investigation on the inheritance.

Patterning of the stay-green behaviour

Two crosses made with PSG16, involving female parents, Pusa 677 and Pusa 44 were both with contrasting duration. The F_1s from both the crosses, Pusa 677/PSG16 and Pusa 44/PSG16 was NSG phenotype. Since the senescence happened within the flowering window of 30 days, there was not much variation for the leaf yellowing pattern between Pusa 677 and Pusa 44, except for the fact that the onset of yellowing in Pusa 44 was slightly delayed than in Pusa 677. In contrast, the progression of yellowing in Pusa 677 was faster. In Pusa 677, the reduction of greenness began onthe 12th day and turned completely yellow by the 23rd day. The PSG16 showed the beginning of chlorophyll depletion after the 20th day and progressed slowly. To assess the pattern of senescence, a five-score colour scale system was developed for visual observations (Fig. 1).

The progress of senescence was markedly denoted by a reduction in greenness and increased yellowing. Since the score pattern was an arbitrary measure, the chlorophyll content was measured indirectly based on the chlorophyll absorbance pattern in a less expensive portable chlorophyll meter, At Leaf® CHL PLUS, which was reportedly an excellent alternative to the expensive devices that are commonly in use (Novichonok et al. 2015; Hebbar et al. 2016) and produced a reliable estimate of chlorophyll content, similar to laboratory estimation (Zhu et al. 2012). These meters emit lights of two wavelengths, 660 nm and 940 nm (infrared) of which the former is absorbed by chlorophyll, while the latter is not. The difference between absorbance and reflectance of the wavelength is measured as 'optical density difference' (ODD) which is converted into chlorophyll content (Monje and Bugbee 1992). The method used was not only non-destructive but also allowed to measure the same leaf continuously over a period. Of the scores, the first two, scores 1 and 2 were associated with SG expression, while scores 3 to 5 were associated with NSG. The progression of senescence was compared to the TCC of the parents during the reproductive phase. The scores were optimized for the chlorophyll depletion pattern which showed a correlation of 0.99 and 0.91 between the score and TCC, in Pusa 677 and PSG16, respectively (Fig. 2a). In both the parents, the senescence progression could be modelled as a function of maturity in days with R^2 values of 0.92 for PSG16 and 0.99 for Pusa 677 (Fig. 2b). Further, the senescence pattern of the parents indicated a clear distinction between them. Pusa 677 began to show significant chlorophyll depletion from the 22nd day after flowering progressing to complete yellowing by the 25th day, while no significant chlorophyll reduction was noticed in PSG16. As per the score scales, PSG16 remained green while Pusa 677 showed complete senescence. A colour graph for the senescence progression based on the chlorophyll depletion is presented in Fig. 2c.



1	2	3	4	5
Leaf remains completely dark green including leaf tip	Leaf turns lighter green, tips are more paler than the rest parts	Tip begins to turn yellow, extending downwards, leaf more yellower, base shows lighter green	Tip turns yellow, or begins to turn brown, leaf generally yellow	Leaf started browning, tip dry and base is turning yellow to brown

Fig. 1. Colour score scale developed for visual scoring of stay-green trait

Inheritance of stay greenness in Pusa stay-green 16 (PSG16)

In both the crosses involving Pusa 677 and Pusa 44, the F_1s manifested NSG trait, indicating the dominance of the trait over the SG. Because of this feature, the F_1s showed a distinct phenotypic appearance distinct from the female parents. The F_1 of Pusa 677/PSG16 showed increased duration along with SG expression. In the case of Pusa 44, the F_1 did not vary as much as that of Pusa 677 but showed distinct SG expression. In the F_2 generation, the SG feature among the progenies showed clear segregation (Table 1). The F_2 segregants of the two crosses (Pusa 677/PSG16 and Pusa 44/PSG16) were classified into different senescence types based on the visual scoring pattern as well as by the total leaf chlorophyll content. The χ^2-test indicated that SG and NSG expression among the progenies segregated for 3:1 ratio.

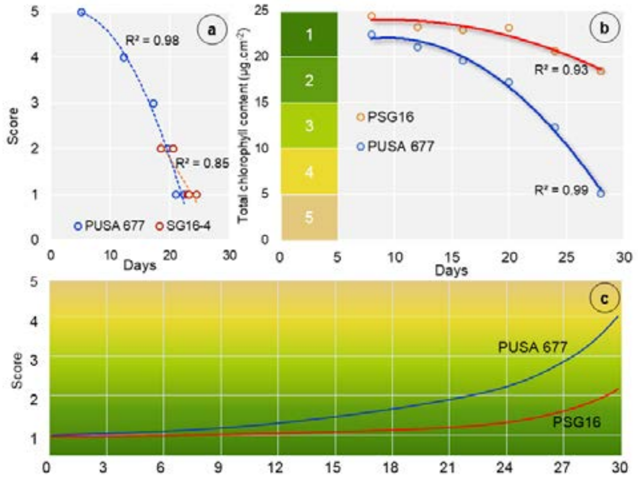


Fig. 2. Pattern of senescence between the genotypes, PSG16 (stay green) and Pusa 677 (non-stay green). (a) Visual score scale showed significant relation with chlorophyll depletion in both the genotypes; (b) progression of chlorophyll depletion during the reproductive phase; (c) a colour graph of senescence pattern among the genotypes.

As per the visual screening and based on the corresponding TCC, in the Pusa 677/PSG16 cross, a total of 82 plants were found similar to PSG16, while 238 plants resembled Pusa 677, the NSG parent. The calculated chi-square value was 0.07 having a high probability of 0.8. The non-significance of deviation from the expected ratio of 3:1 for the monohybrid inheritance indicated stay-green trait in PSG16 follows a 3:1 segregation pattern. In the Pusa 44/PSG16 cross, 61 F_2 plants were SG with a pattern similarto PSG16 while 238 plants were found to show NSG trait as that of Pusa 44, but with varying shades of yellowing. The calculated χ^2-square value in this cross was 1.08 with a probability of 0.3. The segregation of SG in both the crosses showed a similar inheritance pattern that followed a 3:1 ratio, implying that the SG trait in PSG16 was under the influence of a major gene. However, the NSG expression pattern in both the crosses varied, suggesting influence by environmental factors.

Additionally, the segregation pattern supported the conjecture that PSG16 is a novel spontaneous mutant because a recent mutation event can only affect a single locus. If that locus made a major shift in trait expression, the trait would exhibit an inheritance following a monohybrid ratio. In an earlier report, Cha et al. (2002) found that SG trait in a *japonica* rice line, Hwacheong-wx, where single recessive mutant gene, *sgr* on long arm chromosome 9 was found to be responsible for developing a non-functional SG trait, following N-methyl-N-nitrosourea mutagenesis. Although simple inheritance of SG trait in PSG16 identified in this study offers an opportunity for transferring the trait to other backgrounds, a prior establishment of its functionality is to be determined.

SG trait showed influence on agronomic performance

Measuring the average performance of traits is important in any study because the mean and variance are the best way of representing variation present in a population based on first-degree statistics. Since SG trait could be characterized through the chlorophyll depletion pattern, relation of TCC to agronomic features was taken to assess delayed senescence's functionality. We could observe that the average TCC of Pusa 677 (26.1 µg.cm⁻²) was significantly lower than that of PSG16 (36.1 µg.cm⁻²). Drawing a parallel to the parental effects, TCC showed significant variation between SG and NSG groupsacross generations (Table 2). SG progenies had high average TCC values than the NSG types. This indicated that TCC was largely in agreement with the classification for SG behaviour among the progenies. However, in the F_4 generation, the difference between the two groups fell just below the statistical threshold, suggesting either a possible environmental sensitivity of the trait or any deviation in observation time. We rechecked this to find that, in F_4, the chlorophyll measurements were done seven days earlier to physiological maturity, under an unforeseen circumstance. In the F_2 generation, TCC ranged between 12.4 and 48.1 µg.cm⁻², with SG type progenies recording an average of 34.0 µg.cm⁻² as against 28.7 µg.cm⁻² among the NSG types. In F_3, the average for SG types was 35.3 µg.cm⁻², which was higher than the 32.9 µg.m⁻² recorded among the NSG types. Similar observations could be noticed under F_4 and F_5 generations. However, in the F_4 generation, as previously mentioned, the difference was marginal and insignificant. The shrinking range of TCC from generation to generation, also indicated a reducing variability as the generations advanced. The wide range of 6.2 to 46.2 µg. cm⁻² for TCC recorded in the F_2generation could be attributed to a wide spectrum of gene combinations every progeny has inherited along with throwing out of a few transgressive segregants. A similar trend was noticed in F_3, where families showed a narrowing range of 12.4 to 48.1 µg.cm⁻². Among the F_4 and F_5 progenies, the TCC range continued to shrink, with F4 recording a range of 18.1 to 48.5 µg.cm⁻², while F_5 recorded a range between 19.1 and 38.2 µg.cm⁻². The trait range among the segregating generations not only depended on various allelic combinations between parents but also environmental influence (Weinig and Schmitt 2004).

Agronomic performance of the segregating population under different generations was compared for the SG manifestation. The same visual scoring was carried out in each generation along with chlorophyll estimation. The average agronomic performance showed standard distribution for all the traits, indicating quantitative inheritance (Supplementary Fig. 1). The averages of the agronomic traits (Table 3) indicated a comparable performance across generations, with trait ranges exceeding the parental means in both directions. Transgressive segregation is a common feature in segregating populations, which has been known for a long in rice (Ramiah 1933), and occurs due to various genetic interactions, particularly involving complementary

Table 2. One-way ANOVA for total chlorophyll content among the stay-green classes of the cross, Pusa 677/PSG16, for F_2 to F_5 generations

Source		Total chlorophyll content (µg.cm⁻²)			
		F_2	F_3	F_4	F_5
Between group variance		1161.3*	234.0*	93.8^{ns}	354.8*
Within group variance		43.5	32.1	29.3	8.0
F value		26.7	7.3	3.2	44.6
P-value		0.00	0.01	0.08	0.00
SG mean		34.0	35.3	35.2	23.2
NSG mean		28.7	32.9	33.7	26.2
Population mean		29.9	33.5	34.0	27.9
Range		6.2-46.2	12.4-48.1	18.1-48.5	19.1-38.2
Standard error		0.45	0.38	0.36	0.20
Parental means	PSG16 (SG)	36.1	38.1	37.0	30.5
	Pusa 677 (NSG)	23.1	24.6	23.6	25.2
F_1		25.2	-	-	-

* Significant at 5% level; SG, stay green; NSG, non-stay green

Table 3. Phenotypic averages of agro-morphological traits and chlorophyll content at different generations of Pusa 677/PSG16

	Generations					
Trait	P_1	P_2	F_2	F_3	F_4	F_5
PHT	96.4	117.4	91.59±1.18 (70.0-121.0)	92.05±1.27 (55.0-120.0)	89.09±1.48 (63.0-126.0)	92.62±0.9 (75.0-112.0)
PNL	21.3	20.6	23.54±0.32 (17.0-31.0)	23.13±0.29 (15.0-30.0)	22.9±0.3 (18.0-30.0)	21.84±0.27 (16.0-32.0)
NPT	18.8	22.8	12.9±0.64 (3.0-31.0)	13.02±0.51 (5.0-28.0)	10.88±0.29 (6.0-16.0)	13.87±0.26 (10.0-19.0)
SPY	18.2	20.8	19.06±0.52 (8.8-30.4)	18.77±0.55 (6.0-32.4)	15.91±0.84 (3.0-37.0)	22.45±0.8 (12.0-39.0)
GPP	138.6	167.0	157.95±5.14 (86.2-322.6)	159.21±5.43 (89.6-362.4)	163.64±8.92 (59.0-479.0)	159.37±7.12 (67.0-338.0)
SF	84.4	77.3	74.9±0.57 (58.2-85.4)	73.95±0.58 (57.0-84.8)	72.71±1.33 (38.0-93.0)	76.05±1.17 (44.0-94.0)

PHT, plant height in cm; PNL, panicle length in cm; NPT, number of productive tillers; SPY, single plant yield in g; SF, spikelet fertility in %; GPP, grain number per panicle; P_1, Pusa 677; P_2, PSG16

Table 4. Correlation for total chlorophyll content and grain yield in Pusa 677/PSG16 population. Inter-generation correlations for total chlorophyll content are given in upper diagonal and those of grain yield per plant is in lower diagonal. Within generation correlation between the traits is given as diagonal elements (in boldface)

Generations	F_2	F_3	F_4	F_5
F_2	**0.295***	0.034	0.019	0.169*
F_3	0.094	**0.250***	0.616*	0.099
F_4	-0.003	0.051	**-0.007**	0.099
F_5	0.024	0.050	-0.119	**0.286***

*Significant at $p < 0.05$

action of multiple QTLs (Koide et al. 2019; Hagiwara et al. 2006; Xu et al. 1998). Since the chlorophyll content at physiological maturity was found to be the best indicator for SG behavior, the relationship of this trait with grain yield was examined between and within the segregating generations (Table 4). However, correlations between the generations were non-significant for all the traits except for a few cases. However, the insignificance of correlations between generations is not unexpected and is due to continuing trait segregation. Nevertheless, it is noteworthy that the correlation between chlorophyll content and grain yield was consistent and positive within the generations, except for F_4, 0.30 in F_2, 0.25 in F_3 and 0.29 in F_5. The deviation in F_4 was due to the lack of distinction between the SG categories, for reasons already explained. Thus, we could confirm that a consistent relation existed between the SG trait and the grain yield in both the parents and progenies across various generations, indirectly suggesting the functionality of the SG trait in PSG16. However, additional physiological examinations are required to establish the exact functional relations. Based on the inheritance pattern and the consistent yield advantage of the SG type progenies, we concluded that PSG16 was a functional SG parent. Similar observations of high chlorophyll content and yield were reported earlier from the rice variety Swarna, which shows delayed leaf senescence than Moroberekan, a normally senescing genotype (Singh et al. 2020). Thus, a functional SG trait always retained more chlorophyll content during the physiological maturity stage.

Intrageneration intercorrelations of agronomic traits

Since the transgenerational correlations did not show consistency due to ongoing trait segregation, the interrelations were worked between the agronomic traits within each generation (Fig. 3). Plant height consistently exhibited positive relation with panicle length in all the generations. Similarly, single plant yield also showed a consistent association with grains per panicle. The remaining significant relations observed were either not consistent or weak due to several factors. Having delayed leaf senescence during the physiological harvesting stage, PSG16 could be useful in rice breeding for yield improvement, as suggested by the significant positive correlation it had with single plant yield. Ray et al. (1983) could demonstrate a significant yield increase when delayed senescence was induced in Jaya by kinetin application. They could realize, higher mobilization of metabolites in the kinetin-treated plants than the abscisic acid-treated plants that showed enhanced senescence. Retention of chlorophyll content during and beyond the physiological maturity stage, in turn, enhances prolonged photosynthetic agility in plants (Gregersen et al. 2008). Accordingly, PSG16 could be classified as a Type A functional SG type as proposed by Thomas and Howarth (2000). Similar effects were reported by using functional SG genotypes such as Swarna by Singh et al. (2020) and SNU-SG1 by Fu et al. (2011).

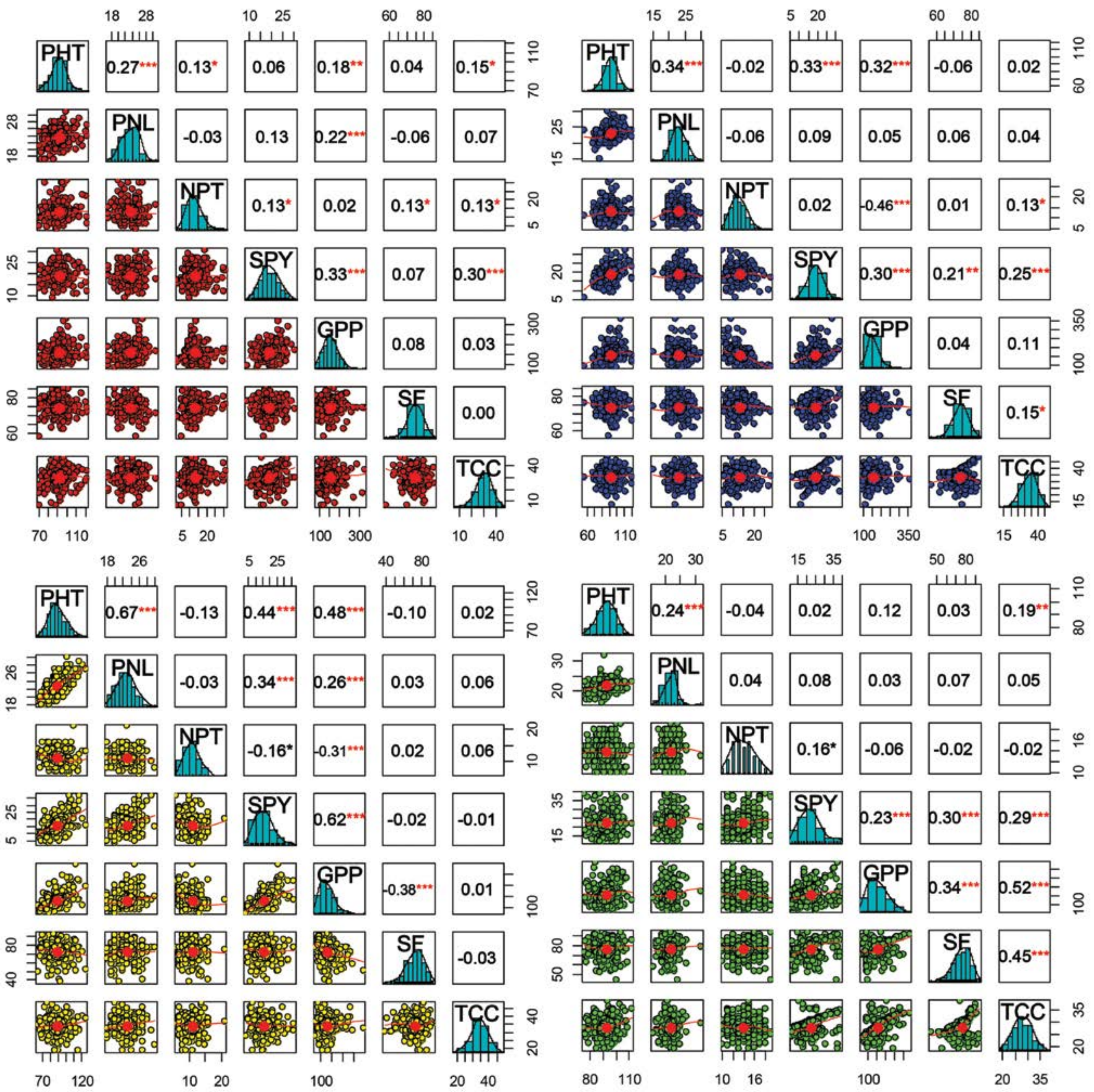


Fig. 3. Correlograms showing the interrelations between agronomic traits and total chlorophyll content (TCC) across four generation of the cross, Pusa 677/ PSG16. *, **, *** represent the significance of correlations at 0.05, 0.01 and 0.001 level, respectively. For agronomic trait abbreviations, please refer Table 3.

Quantitative inheritance

Although the SG trait in the early segregation populations of the cross Pusa 677/PSG16 showed a monohybrid inheritance, its most associated trait, TCC, predominantly showed a quantitative inheritance pattern. Quantitative inheritance occurs when several loci influencing a trait with variable effects segregate in a population throwing out the diverse degree of trait expression, further smoothened by environmental effects (Fisher et al. 1932). Particularly a basic additive model is proposed to understand the combined effect of quantitative genes (Mather 1949). Quantitative variations are further partitioned into gene effects such as dominance, epistasis, etc., based on the generations that are being analyzed. So, for any quantitative trait, it is important to figure out how different genes contribute to the trait expression, in order to determine the most efficient selection and breeding procedure (Sprague 1955; Mangaldeep et al. 2015). A generation mean analysis uses the first-degree statistics of phenotypic distribution such as means and variances to estimate the presence of various gene actions, including their interactions (Innes et al. 1975). Since we are interested in the inheritance pattern of the SG trait in this study, we have analyzed the generation means for the associated trait, TCC. As provided in Table 2., the mean

TCC was high in PSG16; the SG parent designated as P_2 (36.1 µg.cm^{-2}) and low in Pusa 677, the P_1 parent (22.83 µg.cm^{-2}). The F_1s had an intermediate TCC ogf 25.2 µg.cm^{-2}. In the segregation populations, the mean TCC of F_2 was 29.9µg. cm^{-2} and that of F_3 was 33.5µg.cm^{-2}.

Using five generations, the scaling test indicated the significance of non-allelic interactions in the control of TCC (Table 5). Both the scale effects, C and D, showed significance in the respective *t* tests, suggesting the presence of epistasis. Therefore, the inheritance of TCC in the population arising out of the cross Pusa 677/ PSG16 did not fit the simple additive-dominance model. Among these, a greater significance of D than the C advocated a preponderance of additive x additive (*i*) interactions over the dominance x dominance interactions (*l*). Subsequent analysis on gene effects by the five-parameter model for generation means has confirmed this. Among the gene effects tested, all except the *l* component were significant. The mean (*m*) was 29.9 µg.cm^{-2}, the additive effect (*d*) was -4.98 µg.cm^{-2}, while the dominance (*h*) was -13.4 µg.cm^{-2}. Of the interaction components, *i* was significant and high with an estimate of -17.5 µg.cm^{-2}. Despite being in the positive direction, the *l* component (8.2 µg.cm^{-2}) was non-significant. However, the non-significance of dominance x dominance gene effects does not necessarily mean that the interaction component is absent because such results may occur due to cancellation of dominance gene effects at various loci. The opposite sign of *h* and *l* effects further indicated the presence of a duplicate type of gene action in the control of TCC (Kearsey and Pooni 1996). We conclude that TCC is largely under the control of additive genes that are interacting. Among the additive genes, a few may show major effects, with a larger contribution to the trait expression. Besides, the dominance effect was also significant and high, but without interactions. This indicated the presence of a few loci with dominance action. Under this circumstance, a simple selection between the progeny families could result in an early gain. However, procedures such as recurrent selection may be highly useful in effectively consolidating the additive genetic variance. While working with Basmati rice, Bains et al. (1969) advocated repeated backcross as a rewarding approach to pool up the desired genes. Early generation biparental cross followed by selection would also be useful in gathering additive genes, breaking any undesirable linkage they may bring along. Similar gene effects were encountered by Ramli et al. (2016) while studying grain quality parameters in rice. Mgonja et al. (1993) has reported duplicate type of non-allelic interactions for mesocotyl length in rice with a preponderance of additive effect as observed in this study. The minimum number of effective factors (n_E) based on the Castle-Wright estimate was 0.40, and the Burton-Wright estimate was 0.53. However, the estimate seems to be underestimated, because of the violation of certain assumptions such as no interaction between the non-allelic genes (Burton 11951; Burton and Fortson 1966). Non-allelic interactions do exist in the present case, suggesting that TCC is under the control of more than one effective genes, showing epistasis besides additive and dominant effects. Since the dominant gene action was significant, the assessment of the degree of dominance showed a potence ratio of -0.78. Potence ratio ranging between -1 to +1 is suggestive of partial dominance in the genetic control (Ghosh et al. 2018).

Table 5. Scaling test and components of generation means for total chlorophyll content in the population, Pusa 677/ PSG16

Parameters*	Degrees of freedom	Estimate¶	Variance (mean)	Standard Error	t-value
P_1	9	23.88	0.26	0.26	0.51
P_2	9	36.06	0.36	0.33	0.57
F_1	9	25.19	0.23	0.21	0.46
F_2	229	29.86	0.21	0.21	0.45
F_3	229	33.74	0.14	0.14	0.37
C	252	6.91*	5.32	4.72	2.17
D	252	13.06*	4.10	3.59	1.90
m	228	29.86*	0.21	0.21	0.45
d	16	-4.98*	0.27	0.15	0.38
h	464	-13.44*	1.90	1.88	1.37
i	244	-17.51*	2.85	2.25	1.50
l	466	8.19	18.78	18.54	4.31
n_E(CW)	-	0.41			
n_E(BW)	-	0.54			
Potence ratio		0.78			

P_1, Pusa677; P_2, PSG16, F_1 to F_3 are early segregating generations; C and D are test scales; *m*, *d*, *h*, *i* and *l* are genetic effects; * significance at $p < 0.05$; CW, Castle-Wright estimate; BW, Burton-Wright Estimate;¶ Estimates for the generations are the respective population means.

In the current study, the inheritance pattern of SG trait derived from the mutant, PSG16, showed a functional association with grain yield. Although the first filial segregation from the cross Pusa 677/SG16 was suggestive of the presence of a major gene, the associated trait to the SG expression, TCC did show a characteristic quantitative inheritance. The analysis of gene actions governing TCC, showed both additive and dominant gene effects, with dominance having a larger effect than the additive action. This dominance effect could be perceived as assortment of segregating classes in the early segregating generation. However, a preponderance of additive x additive interaction dominated the TCC expression among the progenies. Since the trait offers to delay the senescence in rice, which has the potential to improve yield, a suitable breeding method could be adopted to recruit the SG trait for crop improvement.

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Authors' contribution

Conceptualization of research (KKV); Designing of the experiments (KKV, AR, GKS); Contribution of experimental materials (KKV, AKS, SGK, PKB, MN); Execution of field/lab experiments and data collection (AR, DCV, GKS, VJS, HB, PKB, KNG); Analysis of data and interpretation (KKV, AR); Preparation of manuscript (AR, KKV)

Supplementary materials

Supplementary Fig. S1. Histograms of agronomic traits among four generations of the cross, Pusa 677/PSG16

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Supplementary Figure 1. Histograms of agronomic traits among four generations of the cross, Pusa677/PSG16