Mapping chromosomal regions associated with anther indehiscence with exerted stigmas in CRI-48 and Jasmine 85 cross of rice (Oryza sativa L)

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ABSTRACT
Anther indehiscence in certain wide crosses combines male sterility with stigma exertion, a phenomenon that is desirable for hybrid rice seed production. This study sought to identify chromosomal region(s) that combine anther indehiscence with exerted stigmas. A mapping population consisting of 189 BC1F1 plants was derived from a cross between CRI-48 and Jasmine 85 and backcrossing the resulting F1 to Jasmine 85. Contrary to the three complementary genes mode of inheritance reported earlier, a single locus (AI6-1) was mapped on chromosome 6 at 27.4 cM for anther indehiscence with exerted stigmas through a mixed model-based composite interval mapping (MCIM). This locus was flanked by two single nucleotide polymorphism (SNP) markers, K_ID6002884 and K_ID6003341 within a range of 23.1–28.9 cM. The allele at the locus was contributed by the CRI-48 parent which has Oryza glaberrima ancestry. This locus is suggested to control anther indehiscence and stigma exertion through pleiotropic gene action or cluster of genes.

1. Introduction
Rice (Oryza sativa L.) is a major staple food crop in the developing world (Guimaraes, 2009; Seck et al., 2012). It is cultivated on 11% (156 million ha) of the world’s total arable land second only to wheat in terms of harvested area (FAO, 2017). The demand for rice globally, is predicted to increase as a result of increased growth in population (IRRI, 2010; Seck et al., 2013; Muthayya et al., 2014). Khush (2005) estimates that global production will have to increase by 40% by the year 2030 to meet the growing demand for rice. Genetic improvement of rice has reached a plateau making further increments difficult (Khush, 2005; IRRI, 2010; Khan et al., 2015). Hybrid technology which exploits the phenomenon of heterosis presents a viable means of significantly increasing rice yield than the semi-dwarf inbred varieties currently being utilised (IRRI 1997; Guimaraes, 2009; Fischer et al., 2014; Khan et al., 2015).

Rice, being a strictly self-pollinating crop requires the use of a male sterility system to develop commercial hybrid varieties (Virmani, 1994; Virmani et al., 2003). Cytoplasmic male sterility (CMS) and environment-conditioned genetic male sterility (EGMS) are the two male sterility systems currently available for hybrid rice seed production. The extent and scope of outcrossing determine the ability of these male sterility systems to increase the efficiency of hybrid seed production. Earlier studies have indicated that efficiency of cross pollination in rice is influenced by floral traits including flowering behaviour, pollen longevity, stigma exertion and spikelet opening angle (Virmani, 1994; Takano-Kai et al., 2011). Among these, stigma exertion is the most important trait since it is directly involved in pollination (Virmani, 1994; Takano-Kai et al., 2011; Lou et al., 2014; Bakti and Tanaka, 2019; Xu et al., 2019).

Anther indehiscence, resulting from certain wide crosses, has been suggested as a form of functional male sterility (Sano, 1986; Oka, 1991; Mackawa et al., 1997; Dartey, 2007; Abebrese et al., 2018) with different
modes of inheritance (Cheng and Huang 1986; Sano, 1986; Tamaru, 1991; Maekawa et al., 1997; Darrey, 2007). It has been found to combine male sterility with stigma exertion in specific crosses, a phenomenon believed to adapt the indehiscent plants to outcrossing (Darrey, 2007; Abebrese et al., 2018). This unique combination of anther indehiscence and stigma exertion can present a perfect male sterility system for hybrid rice seed production. The exerted stigmas would trap more pollen from the male parent thereby reducing the pollination barrier often encountered with some cytoplasmic male sterile lines and would increase the hybrid seed set (Virmami, 1994; Takano-Kai et al., 2011).

Recent advances in molecular marker technology through quantitative trait loci (QTL) analysis, allow the identification of chromosomal region(s) underlying important traits in plants (McCouch and Doerge, 1995; Young, 1994; Toure et al., 2000; Collard et al., 2005; Jones et al., 1997, 2009; Nadeem et al., 2018). Breeders can get an insight into the number of loci controlling a trait, their relative importance and approximate positions in the genome (Jones et al., 1997, 2009; Bressghello and Coelho, 2013; Nadeem et al., 2018). Several marker systems are currently available for QTL mapping in plants (Semagn et al., 2006; Collard et al., 2008; Jones et al., 2009; Nadeem et al., 2018). Among these, single nucleotide polymorphism (SNP) markers have emerged as the marker of choice due to their low assay costs, high genomic abundance, locus-specificity, co-dominant inheritance, potential for high throughput analysis and relatively low rates of genotyping error (Semagn et al., 2006, 2014; McCouch et al., 2010, 2013). The continuous progress in high-throughput genomic technologies has led to numerous SNP genotyping platforms that combine a variety of chemistries and allele discrimination techniques (Semagn et al., 2014; Nadeem et al., 2018).

Among these is the competitive allele specific PCR (KASP) (LGc group); a homogenous fluorescence-based genotyping variant of polymerase chain reaction which works based on allele-specific oligo extension and fluorescence resonance energy transfer for signal generation. This has emerged as a more flexible and cost-effective technique with minimal rate of genotyping error (Collard et al., 2008; Semagn et al., 2014; Smith and Moughan, 2015; Steele et al., 2018; Yang et al., 2019).

Over 20 genes have been reported to be involved in regulating anther dehiscence in plants (Keizer, 1987; Goldberg et al., 1993; Matsui et al., 1999; Ma, 2005; Kobayashi et al., 2011; Wilson et al., 2011; Zhou et al., 2011; Peng et al., 2013; Ling et al., 2015; Cardarelli and Costantino, 2018; Estornell et al., 2018; Moon and Jung, 2020). For rice, Zhu et al. (2004) mapped anther indehiscence gene (aid1) on chromosome 6 using a two-element iAc/Ds transposon-tagging system. Using a similar approach, Thangasamy et al. (2011) also found that rice SUMO E3 ligase (str1) gene on chromosome 5 controls spikelet fertility through regulation of anther dehiscence. Anther dehiscence in these two studies (Zhu et al., 2004; Thangasamy et al., 2011) was not associated with stigma exertion, but the genes had pleiotropic effect on other traits. Several studies have also mapped QTLs for stigma exertion on different rice chromosomes (Uga et al., 2003; Miyata et al., 2007; Yan et al., 2009; Li et al., 2014; Lou et al., 2014). Studies on the possible environmental effects on anther indehiscence with exerted stigmas suggested that light, temperature and relative humidity could not modulate the sterility/fertility status of anther indehiscence plants (Zhu et al., 2004; Abebrese et al., 2018; Estornell et al., 2018). Our earlier study (Abebrese et al., 2018) found three complementary genes mode of inheritance for anther indehiscence with exerted stigmas in the CRI-48/Jasmine 85 cross. Information on the chromosomal location of genes controlling anther dehiscence with exerted stigmas is currently lacking. Although it was previously not possible to employ nuclear controlled male sterility in hybrid rice seed production due to the inability to propagate a pure male sterile line, genetic engineering technique now allows constructing useable nuclear male sterile lines for hybrid rice seed production. Therefore, as the first step, this study was carried out to identify chromosomal region(s) controlling anther indehiscence with exerted stigmas in a BC2F1 population of rice.

2. Materials and methods

2.1. Plant material

The parental materials used were two elite rice genotypes, CRI-48 (female) and Jasmine 85 (male). CRI-48 is an interspecific stabilized breeding line developed at the Council for Scientific and Industrial Research · Crops Research Institute (CSIR-CRI), Fumesoa, Ghana, from the cross IDSA 85 × NERICA 1 (Figure 1). It has dehiscent anthers and non-exerted stigmas. Jasmine 85 is a fragrant indica variety which was developed at the International Rice Research Institute (IRRI) as IR841, from the cross IR262 × Khao Dawk Mali 105. It was released in the USA in 1989 as Jasmine 85 (Bollich, 1989; Asante, 2012). It was subsequently released as a commercial variety in Ghana in 2009 and for some time, was the most widely grown variety in Ghana because of its good taste, soft texture and fragrance (Asante et al., 2013; Ragassa et al., 2013). Jasmine 85 also has dehiscent anthers and non-exerted stigmas. The F1 progeny resulting from the cross between CRI-48 and Jasmine 85 exhibited anther indehiscence with exerted stigmas as observed in our previous study (Abebrese et al., 2018).

2.2. Developing the mapping population

Jasmine 85 was crossed to CRI-48 between July and October, 2013 at Nyankpala, Northern Ghana (09° 24’ 17.8” N, 00° 57’ 57.0” W, 143 m). The resultant F1 plants were raised in buckets. A single F1 plant was backcrossed to Jasmine 85 at the same location between July and October 2014. The 189 BC1F1 seeds were planted in buckets to raise 189 BC2F1 progenies which served as the mapping population for the present study (Figure 2).
2.3. Genotyping of the mapping population

Using a disc puncher, leaves (of 6mm diameter) were sampled from the two parents, four F1 plants and 189 BC1F1 plants three weeks after sowing and sent to LGC genomics, UK for DNA extraction and SNP genotyping. DNA extraction and KASP genotyping assay were carried out as described by Smith and Moughan (2015). The two parents were first screened with a total of 1885 SNP markers (LGC group) for polymorphism out of which 849 were polymorphic. Out of the 849 identified polymorphic markers, 246 evenly-spaced markers with known mapped positions were selected for genotyping the mapping population.

2.4. Phenotyping for anther indehiscence and stigma exertion

The phenotyping experiment was carried at Nyankpala, in the Guinea Savanna ecology of Northern Ghana (09° 24’ 17.8” N, 00° 57’ 57.0” W, 143 m). The seeds of the BC1F1 plants were pre-germinated in white tissue paper for four days and the resulting seedlings were nursed in buckets for 21 days followed by transplanting of one plant per 12 L bucket. Individual plants were provided with 8g of N.P.K. (15-15-15) fertilizer three weeks after transplanting, 4g of Ammonium sulphate at panicle initiation and watered whenever necessary. All other standard agronomic practices were followed as recommended. Individual plants were then phenotyped for the expression of anther indehiscence and stigma exertion. Dehiscence/indehiscence status of individual plants was scored by gently tapping panicles of individual plants at anthesis and visually observing extent of released pollen which was visible to the naked eye (Dartey, 2007). Absence of dehisced pollen was further checked with a hand lens to be sure that anthers remained indehiscent until drying up. Individual plants were scored for dehiscence/indehiscence of anthers and exerted/non-exerted of stigmas. Plants with dehiscent anthers and non-exerted stigmas were assigned zero (0) whereas their indehiscent counterparts with exerted stigmas were assigned one (1) for analysis.

2.5. Linkage map construction and QTL analysis

The genotyping data was used to construct a genetic linkage map for the CRI-48/Jasmine 85//Jasmine 85 BC1F1 population using QTL Network software v2.1 (Yang et al., 2007), a mixed model-based composite interval mapping (MCIM), based on default parameters of a 1000 permutation time, walk speed of 1cM, testing and filtration windows of 10cM each and a putative QTL detection at 0.05 significance level. MapChart Version 2.3 (Voorrips, 2002) was used for the construction of detailed linkage map showing the position of the QTL. The gene nomenclature followed that of McCouch et al. (1997) where a 2- or 3-letter abbreviation is followed by the number of chromosome on which the
### Table 1. Anther indehiscence and stigma exertion status of BC1F1 plants.

| Plant No. | Anther dehiscence status | Stigma exertion status | Plant No. | Anther dehiscence status | Stigma exertion status | Plant No. | Anther dehiscence status | Stigma exertion status | Plant No. | Anther dehiscence status | Stigma exertion status |
|-----------|--------------------------|------------------------|-----------|--------------------------|------------------------|-----------|--------------------------|------------------------|-----------|--------------------------|------------------------|
| Plant 1   | Dehisce                  | Non exerted            | Plant 26  | Dehisce                  | Non exerted            | Plant 51  | Indehisce                | Exerted                | Plant 76  | Indehisce                | Exerted                |
| Plant 2   | Indehisce                | Exerted                | Plant 27  | Dehisce                  | Non exerted            | Plant 52  | Indehisce                | Exerted                | Plant 77  | Dehisce                  | Non exerted            |
| Plant 3   | Indehisce                | Exerted                | Plant 28  | Indehisce                | Exerted                | Plant 53  | Dehisce                  | Non exerted            | Plant 78  | Indehisce                | Exerted                |
| Plant 4   | Dehisce                  | Non exerted            | Plant 29  | Indehisce                | Exerted                | Plant 54  | Indehisce                | Exerted                | Plant 79  | Indehisce                | Exerted                |
| Plant 5   | Indehisce                | Exerted                | Plant 30  | Dehisce                  | Non exerted            | Plant 55  | Indehisce                | Exerted                | Plant 80  | Indehisce                | Exerted                |
| Plant 6   | Dehisce                  | Non exerted            | Plant 31  | Dehisce                  | Non exerted            | Plant 56  | Indehisce                | Exerted                | Plant 81  | Indehisce                | Exerted                |
| Plant 7   | Indehisce                | Exerted                | Plant 32  | Indehisce                | Exerted                | Plant 57  | Indehisce                | Exerted                | Plant 82  | Indehisce                | Exerted                |
| Plant 8   | Indehisce                | Exerted                | Plant 33  | Indehisce                | Exerted                | Plant 58  | Dehisce                  | Non exerted            | Plant 83  | Indehisce                | Exerted                |
| Plant 9   | Indehisce                | Exerted                | Plant 34  | Indehisce                | Exerted                | Plant 59  | Indehisce                | Exerted                | Plant 84  | Indehisce                | Exerted                |
| Plant 10  | Dehisce                  | Non exerted            | Plant 35  | Dehisce                  | Non exerted            | Plant 60  | Indehisce                | Exerted                | Plant 85  | Indehisce                | Exerted                |
| Plant 11  | Indehisce                | Exerted                | Plant 36  | Dehisce                  | Non exerted            | Plant 61  | Indehisce                | Exerted                | Plant 86  | Indehisce                | Exerted                |
| Plant 12  | Indehisce                | Exerted                | Plant 37  | Indehisce                | Non exerted            | Plant 62  | Dehisce                  | Non exerted            | Plant 87  | Indehisce                | Exerted                |
| Plant 13  | Indehisce                | Exerted                | Plant 38  | Indehisce                | Exerted                | Plant 63  | Indehisce                | Exerted                | Plant 88  | Indehisce                | Exerted                |
| Plant 14  | Indehisce                | Exerted                | Plant 39  | Dehisce                  | Non exerted            | Plant 64  | Indehisce                | Exerted                | Plant 89  | Indehisce                | Exerted                |
| Plant 15  | Indehisce                | Exerted                | Plant 40  | Indehisce                | Exerted                | Plant 65  | Indehisce                | Exerted                | Plant 90  | Indehisce                | Exerted                |
| Plant 16  | Indehisce                | Exerted                | Plant 41  | Indehisce                | Exerted                | Plant 66  | Dehisce                  | Non exerted            | Plant 91  | Indehisce                | Exerted                |
| Plant 17  | Indehisce                | Exerted                | Plant 42  | Indehisce                | Exerted                | Plant 67  | Indehisce                | Exerted                | Plant 92  | Indehisce                | Exerted                |
| Plant 18  | Indehisce                | Exerted                | Plant 43  | Indehisce                | Exerted                | Plant 68  | Indehisce                | Exerted                | Plant 93  | Indehisce                | Exerted                |
| Plant 19  | Indehisce                | Exerted                | Plant 44  | Dehisce                  | Non exerted            | Plant 69  | Indehisce                | Exerted                | Plant 94  | Indehisce                | Exerted                |
| Plant 20  | Indehisce                | Exerted                | Plant 45  | Indehisce                | Exerted                | Plant 70  | Indehisce                | Exerted                | Plant 95  | Indehisce                | Exerted                |
| Plant 21  | Dehisce                  | Non exerted            | Plant 46  | Indehisce                | Exerted                | Plant 71  | Indehisce                | Exerted                | Plant 96  | Dehisce                  | Non exerted            |
| Plant 22  | Indehisce                | Exerted                | Plant 47  | Indehisce                | Exerted                | Plant 72  | Indehisce                | Exerted                | Plant 97  | Indehisce                | Exerted                |
| Plant 23  | Dehisce                  | Non exerted            | Plant 48  | Indehisce                | Exerted                | Plant 73  | Indehisce                | Exerted                | Plant 98  | Indehisce                | Exerted                |
| Plant 24  | Indehisce                | Exerted                | Plant 49  | Dehisce                  | Non exerted            | Plant 74  | Indehisce                | Exerted                | Plant 99  | Indehisce                | Exerted                |
| Plant 25  | Indehisce                | Exerted                | Plant 50  | Indehisce                | Exerted                | Plant 75  | Indehisce                | Exerted                | Plant 100 | Indehisce                | Exerted                |

(continued on next page)
QTL is located and a terminal suffix, separated by a period, provides a unique identifier to distinguish multiple QTL on a single chromosome.

3. Results

3.1. Distribution of anther indehiscence and stigma exertion

The anther indehiscence trait was exhibited only by the F1s and subsequent generations of the CRI-48/Jasmine 85 cross but not their individual parents. Both CRI-48 and Jasmine 85 had dehisced anthers with non-exerted stigmas. All the F1 plants from the CRI-48/Jasmine 85 cross exhibited anther indehiscence with exerted stigmas (Figure 3). The BC1F1 plants segregated for anther dehiscence/indehiscence and stigma exertion/non-exertion. Out of the 189 BC1F1 plants scored for the mapping study, 38 had dehiscent anthers whereas 151 had indehiscent anthers (Table 1). Thirty-eight (38) plants had their stigmas not exerted whereas 151 plants had their stigmas exerted (Table 1). Florets with indehiscent anthers always had their stigmas exerted outside the hull whilst stigmas were enclosed within the hull for florets with dehiscent anthers (Table 1). The two parents also differed in many agro-morphological traits including days to flowering, basal pigmentation and grain length. Whereas Jasmine 85 flowered within 85 days, CRI-48 flowered at 70 days. The BC1F1 plants showed variations and segregated for the various agro-morphological traits. Temperature at flowering did not have any effect on the expression of anther indehiscence.

3.2. Genetic analysis and QTL detection

A genetic linkage map with 12 linkage groups corresponding to the 12 gametic rice chromosomes was constructed, spanning a total length of 1520.2 cM at an average marker interval of 6.18 cM (Table 2) using 246 markers. Chromosome 1 was the longest (179.4 cM) and had 40 markers with an average marker density of 4.49 cM. Chromosome 9 spanned 98.6 cM and was the shortest with average marker density of 7.58 cM. Summary of marker positions on the genetic linkage map is presented in Table 2. A single locus (AI6-1) was mapped at 27.4 cM on chromosome 6 for anther indehiscence with exerted stigmas. This locus was flanked by K_ID6002884 and K_ID6003341 within a range of 23.1–28.9 cM (Table 3; Figure 4). The allele at this locus was contributed by the CRI-48 parent which has Oryza glaberrima ancestry (Table 3).

4. Discussion

This study was set out to preliminarily map the chromosomal locations controlling anther indehiscence with exerted stigmas in rice for further studies on fine mapping and cloning the underlining gene(s). The underlying gene(s) could possibly be manipulated through marker assisted selection (MAS) or genetic engineering to develop male sterile rice lines with enhanced outcrossing for future hybrid rice seed production. The study followed the bi-parental mapping procedure.

Diverse parents are in vogue recommended for bi-parental QTL mapping studies to enable high marker polymorphism detection and adequate variation within the trait of interest (Collard et al., 2008; Jones et al., 1997, 2009). The presence of 849 polymorphic markers, representing 45% of the total 1885 SNP markers from the initial polymorphism survey suggests that the two parents were different in most of their genomic regions. This was likely because Jasmine 85 (the male parent) is an indica variety whereas the CRI-48 parent (the female parent) is from an interspecific japonica/NERICA cross with O. glaberrima parentage (Somado et al., 2008). A high-density genetic linkage map with evenly distributed markers is a prerequisite for identifying chromosomal regions that contain genes of interest using QTL analysis (McCouch and Doerge, 1995; Bernardino, 2008; Collard et al., 2008). A map length of 1520.2 cM generated from the 246 evenly distributed SNP markers was similar in length to linkage maps constructed using simple sequence repeat (SSR), restriction fragment length polymorphism
(RFLPs) and amplified fragment length polymorphism (AFLPs) markers (Lanceras et al., 2000; Temnykh et al., 2000; Collard et al., 2008). An average marker density of 6.18 cM for the constructed map was appropriate for initial QTL detection. Bernardo (2008) recommended average marker density of <10 cM for such purposes.

Expression of anther indehiscence only by the F1s but not their individual parents suggests that the trait might be as a result of complementary genes from the two parents. Different modes of inheritance have been reported for anther indehiscence from different cross combinations (Sano, 1986; Maekawa et al., 1997; Dartey, 2007). Our earlier study (Abebrese et al., 2018) found anther indehiscence with exerted stigmas in the CRI-48/Jasmine 85 cross to conform to the three complementary genes mode of inheritance reported by Dartey (2007). However, using genome-wide SNP markers, a single locus (AI6-1) was mapped for anther indehiscence with exerted stigmas in this current study. It could be that, the three complementary genes suggested by conventional genetic analysis are in a cluster. Fine mapping using denser molecular markers could reveal more in this direction. Segregation of anther indehiscence in the mapping population was skewed and did not fit into any of the earlier reported ratios (Sano, 1986; Maekawa et al., 1997; Dartey, 2007). Failure of the segregating pattern of the mapping population to conform to the 7:1 (indehiscence: dehiscence) mode of inheritance reported earlier

| Chromosome | Length (cM) | Number of SNP makers | Average marker density (cM) |
|------------|-------------|----------------------|---------------------------|
| 1          | 179.4       | 40                   | 4.49                      |
| 2          | 142         | 25                   | 5.68                      |
| 3          | 160.4       | 22                   | 7.29                      |
| 4          | 114.6       | 22                   | 5.21                      |
| 5          | 132.1       | 23                   | 5.74                      |
| 6          | 122.3       | 24                   | 5.1                       |
| 7          | 108         | 22                   | 4.91                      |
| 8          | 130.2       | 17                   | 7.66                      |
| 9          | 98.6        | 13                   | 7.58                      |
| 10         | 100.9       | 11                   | 9.17                      |
| 11         | 114.4       | 14                   | 8.17                      |
| 12         | 117.3       | 14                   | 8.38                      |
| Total/Average | 1520.2       | 246                  | 6.18                      |

| Locus | Chr. | Interval | position | range | A   | SE   | P-Value | Source of allele |
|-------|------|----------|----------|-------|-----|------|---------|------------------|
| AI6-1 | 6    | K_ID6002884-K_ID6003341 | 27.4 | 23.1–28.9 | -0.8388 | 0.0793 | 0.00001 | CRI-48 |

Figure 4. Genetic linkage map showing the locus (AI6-1) mapped for anther indehiscence with exerted stigmas on chromosome 6 between SNP markers K_ID6002884 and K_ID6003341.

Table 2. Summary of genetic linkage map for the 246 SNP markers.

Table 3. Information on the locus identified for anther indehiscence with exerted stigmas.
could be due to the smaller population size. Also, hybridity of individual BC$_1$F$_1$ plants was mostly established by phenotypically examining the plants to confirm combination of unique traits of the two parents. Few plants which lacked such clear trait combinations were discarded. Such minor selection might have also contributed to the segregation distortion observed in the mapping population.

The locus for anther indehiscence with exerted stigmas in this study was mapped to 27.4 cm on chromosome 6. This locus was flanked by K$_{1}$ID6002884 and K$_{1}$ID6003341 within a marker interval of 23.1–28.9 cm Zhu et al. (2004) identified a rice (Oryza sativa L. cv Nipponbare) recessive mutant, anther indehiscence (aid1) gene, through the reverse genetics approach (a two-element iAc/iDs transposon-tagging system), showing partial to complete spikelet sterility. The aid1 gene which was mapped to 13.5 cm (124,000–140,000 bp) on chromosome 6 is about 13.9 cm away from the locus mapped in this present study. Among the several QTLs reported for stigma exertion of rice (Uga et al., 2003; Miyata et al., 2007; Yan et al., 2009; Lou et al., 2014), two (qPDES-6 and qPES-6) have been mapped on chromosome 6 (Lou et al., 2014). These two QTLs were flanked by simple sequence repeat (SSR) markers RM8225 and RM225 within an interval of 26.2–54.1 cm (3,416,523–9,309,118 bp, Nipponbare sequence 2009, www.gramene.org) on chromosome 6. The locus for anther indehiscence with exerted stigmas in this present study which was mapped within 23.1–28.9 cm is in the range reported by Lou et al. (2014). Florets with indehiscent anthers always had their three stigmas exerted outside the hull whereas stigmas were enclosed within the hull for florets with dehisced anthers. Anther indehiscence always co-segregated with stigma exertion in a 964 BC$_1$F$_1$ segregating population reported by Abebrese et al. (2018) and that of a 517 reported by Dartey (2007). Therefore, it seems the single locus (AI6-1) controls anther indehiscence and stigma exertion pleiotropically. The aid1 gene reported by Zhu et al. (2004) had a pleiotropic effect on tillering and flowering time. Presence of pleiotropy could aid in manipulating the two traits together to design a useful male sterility system with enhanced outcrossing.

Review of literature suggests two sources of anther indehiscence genes. Anther indehiscence could originate from a single rice genotype or species (Cheng and Huang 1980; Sano, 1986; Li et al., 2011). For instance, Sano (1986) suggested a dominant gene (W020) from O. glaberrima as responsible for anther indehiscence. Cheng and Huang (1980) also traced anther indehiscence genes to O. rufipogon. Alternatively, anther indehiscence could also be as a result of complementary action of genes from two genotypes or species (Maekawa et al., 1997; Dartey, 2007). Maekawa et al. (1997) suggested that anther indehiscence is controlled by complementary action of three dominant genes. In their study, cv. Silewah (one of the parents for their mapping population) putatively had one of the three genes and cv. Hayakogane (the other parent) had the other two. Dartey (2007) also postulated involvement of three complementary genes to control anther indehiscence. Anthers dehisce if all three genes exist in the homozygous state, but indehiscence would result if one, two or all three genes exist in the heterozygous state. The allele at the mapped locus for this current study was contributed by the CR-48 parent. The CRI-48 has a glaberrima ancestry from its NERICA parent. The source of the anther indehiscence gene(s) could possibly be traced to this glaberrima parent. Anther indehiscence has also been reported as a common phenomenon in glaberrima-sativa crosses and was attributed to chromosomal aberrations (Sano, 1986).

5. Conclusion

The study identified a single mapped locus between SNP markers K$_{1}$ID6002884 and K$_{1}$ID6003341 on chromosome 6 for anther indehiscence with exerted stigmas. The allele at this locus was contributed by the CRI-48 parent which has Oryza glaberrima ancestry. We suggest that this locus controls anther indehiscence and stigma exertion through pleiotropic gene action or the three complementary genes might be in a cluster. Fine mapping with denser molecular markers could help uncover the underlying gene(s).

Declarations

Author contribution statement

Samuel Oppong Abebrese: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nana Kofi Abaka Amoah: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Paul Kofi Ayirebi Dartey: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Isaac Kofi Bimpong: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Richard Akromah: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Vernon Edward Gracen; Samuel Kwame Offei; Eric Yirenkyi Danquah: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article supplementary material/referenced in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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