

Evaluation of polymorphisms among South African winter wheat varieties

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Abstract

Wheat production globally is constrained by among other factors stem rust. Stem rust is a major disease of wheat that can result in up to 100% yield losses. Though stem rust had been managed before using host resistance genes, most of these genes have been overcome by emergence of new and virulent stem rust races such as Ug99. The stem rust race Ug99 is virulent against most commonly used stem rust resistance genes and currently about 85-95% of wheat varieties are reported to be susceptible. At present, 13 variants of Ug99 that differ in virulence have been reported in 13 countries. *In South Africa, a number of winter varieties have been shown to possess resistance against some stem rust race. However, the genes conferring resistance in these varieties are not known.* Therefore, new sources of resistance should be explored and deployed in commercial varieties. The objective of this study was to screen for polymorphisms among different parental lines. Eight stem rust resistant winter wheat varieties and a single susceptible spring wheat variety were screened for polymorphisms with 42 SSR markers. Results show presence of variation among parental lines hence suitable for mapping of stem rust resistance.

Key words: Genes, markers, polymorphism, resistance, stem rust, winter wheat

Résumé

La production de blé à l'échelle mondiale est limitée par d'autres facteurs parmi lesquels on trouve la rouille de la tige. La rouille de la tige est une maladie importante de blé qui peut entraîner jusqu'à 100 % de perte de rendement. Bien que la rouille ait été gérée avant d'utiliser des gènes de résistance de l'hôte, la plupart de ces gènes ont été surmontés par l'émergence de nouvelles et virulentes races souches de rouille tels que Ug99. La race de la rouille de la tige Ug99 est virulente contre des gènes de résistance souches de rouille les plus couramment utilisés et actuellement environ 85-95% des variétés de blé sont signalés à être sensibles. A l'heure actuelle, 13 variantes de Ug99 qui diffèrent dans la virulence ont été signalés dans 13 pays. *En Afrique du Sud, un certain nombre de variétés d'hiver ont été démontré qu'elles possèdent une résistance contre une race de rouille de tige. Cependant, les gènes conférant une résistance à ces variétés ne sont pas connus.* Par conséquent, de nouvelles sources de résistance devraient être explorés et déployés dans les

variétés commerciales. L'objectif de cette étude était de dépister les polymorphismes entre les lignées parentales différentes. Huit variétés résistantes à la rouille de la tige de blé d'hiver et une seule variété de blé de printemps sensibles ont été criblées pour des polymorphismes avec 42 marqueurs SSR. Les résultats montrent la présence de variation entre les lignées parentales, donc appropriés pour faire la cartographie de la résistance à la rouille de la tige.

Mots clés: gènes, marqueurs, polymorphisme, résistance, rouille de la tige, le blé d'hiver

Background

Wheat is the third most important grain cereal crop in the world (FAOSTAT, 2013). It provides 30% edible dry matter and 60% daily calories in many low-income countries (FAOSTAT, 2015). As the world population continues to increase, it is predicted that a 60% increase in wheat production will be adequate for the demands of developing countries up to 2050 (Rosegrant and Agcaoili, 2010). Wheat production has been increasing over the past few years but it is currently constrained by many factors like low soil fertility, droughts and rusts. These biotic and abiotic factors remain a great hindrance in achieving the food sufficiency goal (Guotam *et al.*, 2015). Stem rust or black rust is one of the most destructive diseases of wheat causing up to 100% yield losses during epidemics (Hodson, 2011). This disease is well known historically as the most feared in wheat growing regions of all continents (Singh *et al.*, 2011). Roelfs *et al.* (1992) and Mamo *et al.* (2014) reported that hexaploid common bread wheat (*Triticum aestivum* L.), tetraploid durum wheat (*T. turgidum* var. *durum*), barley (*Hordeum vulgare* L.), triticale (X Triticosecale) and wheat progenitors are major hosts of the stem rusts. *Barberies vulgaris* L. is the alternate host for the stem rust fungus and hence the production of new virulent pathotypes (Leonard and Szabo, 2005; Todorovska *et al.*, 2009; Upadhyaya *et al.*, 2015). This pathogen affects all parts of the wheat plant (Spanic *et al.*, 2015). Controlling stem rusts by deploying resistant cultivars has been the most economic and environmentally viable approach (Gao *et al.*, 2015; Kumssa *et al.*, 2015). Before 1999, stem rust disease outbreaks globally were rare except in Ethiopia where a major stem rust epidemic devastated the widely grown wheat cultivar Enkoy in 1993 and 1994 (Singh *et al.*, 2011). Currently thirteen variants of Ug99 have been identified with different virulences for *Sr9h*, *Sr21*, *Sr24*, *Sr31*, *Sr36* and *SrTmp* (Singh *et al.*, 2011, 2015; Pretorius *et al.*, 2012), in 13 countries (Visser *et al.*, 2011; Pretorius *et al.*, 2012). To mitigate the effects of stem rust in wheat, it is therefore vital to search for new sources of resistance. Therefore, the purpose of this study was to evaluate the polymorphisms among the different wheat parental lines and identify SSR markers for mapping of stem rust resistance genes.

Literature summary

Stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.), or *Pgt* is an economically destructive disease of wheat globally (Ghazvini *et al.*, 2012) and it has been reported to cause substantial yield losses of even up to 100% during epidemics (Hodson, 2011). During the middle of the 20th century eastern and central Europe (Zadoks, 1963) and other countries

such as Australia, China and India recorded yield losses of 20-30% due to stem rust (Leonard and Szabo, 2005). Even if the deployment of resistant cultivars has been recommended as the most economic and environmentally friendly means of controlling the stem rust epidemics (Balgain *et al.*, 2016). With the emergence of a new stem rust race Ug99 with virulence against previously most widely efficient stem rust resistance genes like *Sr31* and *Sr38* and with about only 5-15% of wheat varieties reported to be resistant to Ug99 (Mago *et al.*, 2011; Singh *et al.*, 2015). Though the number of identified stem rust resistance genes has increased by at least 10 in the last four years (Pumphrey, 2012), *Pgt* continues to evolve as was recently confirmed by emergence of a new virulent non-Ug99 TKTTF race in Ethiopia that caused up to 100% yield losses in Digalu which was one of most used cultivars (Olivera *et al.*, 2012, 2015; Singh *et al.*, 2015). According to Hodson (2011), *Pgt* is known to be carried by wind for long distances and mutate into new and virulent races. Wind trajectory studies have predicted the likely establishment of these new virulent races in major wheat growing areas of the world (Hudson, 2011). Virulent stem rust races such as TTKSK have been effectively controlled with fungicide applications, this approach is costly and the negative effects of these chemicals on the environment merit the use of host plant resistance genes (Wanyera *et al.*, 2009; Mamo *et al.*, 2014). Thus, discovery and deployment of new sources of resistances to stem rust should be explored, such as wild relatives (Balgain *et al.*, 2015, 2016; Guerrero-Chavez *et al.*, 2015). With limited number of stem rust resistance genes in commercial bread wheat, many varieties remain susceptible to stem rust (Singh *et al.*, 2011, Yu *et al.*, 2015). New virulent pathogen variants can evolve and defeat individual resistance genes (Pretorius *et al.*, 2012; Pujol *et al.*, 2015), therefore to deploy stem rust resistance genes into new wheat cultivars, it has been shown that combining many stem rust genes can result in enhanced durability of resistance (Singh *et al.*, 2011; Yu *et al.*, 2014).

However, combining various alien genes into one variety increases the proportion of foreign chromosomal segments that leads to potential negative effects of reduced yield and end-use quality (Liu *et al.*, 2013; Yu *et al.*, 2015). This can be mitigated by combining effective genes from the wheat primary gene sources that rarely introduce negative linkage drags effects (Balgain *et al.*, 2015; Guerrero-Chavez *et al.*, 2015; Yu *et al.*, 2015). The conventional gene combining method is difficult and tedious as simultaneous tests of the same wheat cultivar with many different rust races have to be conducted prior to cultivar selection (Haile and Röder, 2013) and because it is often difficult for scientists to keep all important rust races needed for rust evaluations besides quarantine races cannot be tested (Wu, 2008). Hence an effective and efficient alternative is to use molecular markers to combine 2-3 genes and achieve durable resistance (Haile and Röder, 2013). Molecular markers that are closely linked to rust resistance genes can be used to improve the selection efficiency of wheat breeding programmes (Todorovska *et al.*, 2009). Therefore, identifying these molecular markers can result in rapid incorporation of multiple resistance genes in wheat breeding lines (Bernardo *et al.*, 2012; Pumphrey, 2012; Lopez-Vera *et al.*, 2014, Dunckel *et al.*, 2015). In South Africa, a number of winter wheat varieties have been shown to possess resistance against some races of stem rust. However, genes conferring resistance in these varieties are not known (Figlan *et al.*, 2014).

Study description

Development of a mapping population. Eight plant populations involving crosses of resistant cultivars and a susceptible cultivar (Line 37) were grown in the screen house from March- August 2016. Ten plants of each F_1 were planted in 2 l containers and selfed to produce a segregating F_2 population. A bout 10 seeds of each F1 plant per cross were used to develop the F_2 population. This yielded 200 F_2 seeds per population to be evaluated alongside their parental lines for mapping stem rust resistance. Forty-two SSR markers were screened on eight stem rust resistant South African winter wheat cultivars and one susceptible spring wheat line for polymorphisms.

Results and discussion

The results show that of the 42 SSR markers, 73.8% were polymorphic in different winter wheat varieties, 26.2% were monomorphic in all the eight wheat varieties while 28.6% were polymorphic in all the eight wheat varieties. Within variety polymorphic loci varied from 0 to 69.05% and similarly Colomba and Gregorini (2011) obtained 0 to 57.14% variation for polymorphic loci within durum wheat in Italy. Principal component analysis (PCA) of genetic distance among varieties showed the first two axes accounting for 65.6% of the total variability compared to 61.60% (Colomba and Gregorini, 2011). The most diverse wheat variety was SST 387 (69.05%) whereas Komati was the least diverse as compared to the susceptible spring wheat variety at 50.0%. SST 387 is the most promising variety to be used in crosses with susceptible variety and to track the segregation of stem rust resistance gene(s). The low levels of polymorphisms among the eight varieties is due to the fact that

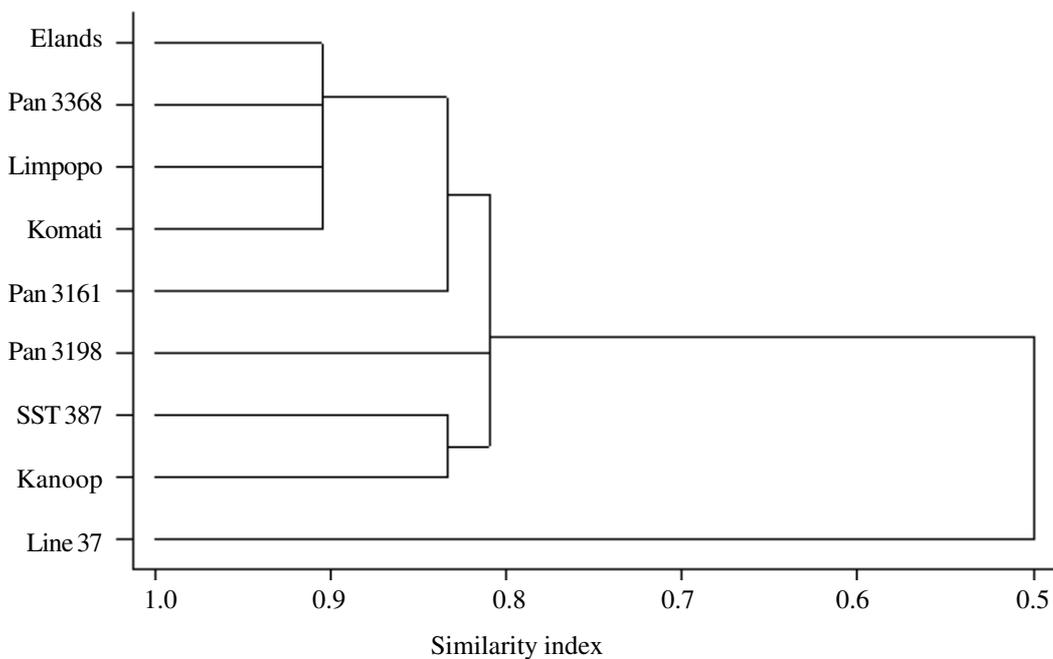


Figure 1. Dendrogram showing the genetic diversity among eight winter wheat varieties

wheat is self-pollinated crop, while the difference between the spring and winter wheat is due to their different genetics. Cluster analysis of SSR datasets assigned the winter wheat varieties into three major classes such class I (SST 387 and Kanoop), class II (Pan 3198), class III (Pan 3161, Komati, Limpopo, Pan 3368 and Elands).

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