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Review

Phenolics and their potential as biochemical markers for wheat rust and Russian wheat aphid resistance in South Africa

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Three types of wheat rusts and Russian wheat aphid (RWA) are important constraints to wheat production in South Africa. Genetic resistance provides an effective and safe option to control these pests. However, breeding for resistance to rusts and RWA in South Africa largely depends on screening thousands of germplasm in the field at several localities. The success of such trials depends on optimum development of diseases and insects, which is mostly difficult to achieve due to seasonal variations in climatic conditions. Therefore, there is a need to improve this laborious and time-consuming screening method. Protective plant phenols, which are involved in resistance to biotic factors, are gaining more attention from plant breeders as potential biochemical markers. Such markers assist in overcoming the above limitations by allowing accurate and faster selection of resistant materials. For example, higher levels of phenolic compounds such as phytoalexins have been observed in resistant than in susceptible wheat cultivars suggesting that phenols may possibly be used as biochemical markers. This review paper discusses the different types of phenols, their significance in resistance to biotic factors and their potential application in breeding for resistance to wheat rusts and insects in South Africa.

Keywords: Phenolics, disease, resistance, breeding, South Africa.

INTRODUCTION

Stem rust (black rust), leaf rust (brown rust) and stripe rust (yellow rust), caused by *Puccinia graminis* f. sp *tritici* (Pgt), *P. triticina* (Pt) and *P. striiformis* f. sp *tritici* (Pst), respectively, can cause significant grain yield losses in wheat (Singh et al., 2015; McIntosh et al., 1995). Of the three rusts, Pgt, and in particular the highly virulent strain Ug99, first detected in Uganda in 1999, is of concern to breeders, owing to its ability to spread rapidly and cause extensive losses in wheat production, resulting in a high risk to food security worldwide (Ellis et al., 2014; Singh et al., 2011; Pretorius et al., 2000). All three wheat rusts are important diseases affecting bread wheat in South Africa. Leaf and stem rust are more frequent in the winter rainfall regions of the Western Cape Province, whereas yellow rust is more important in the cool winter wheat production regions such as the Free State Province (Terefe et al., 2016, 2014; Pretorius et al., 2007). Rust infection in cereals can be controlled by using chemicals and resistant cultivars, with the latter having advantages for environmental and economic reasons.

Aphids are known to be the largest group of phloem-feeding insects, and their enormous reproductive

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potential that allows quick infestation of their host makes them one of the most devastating pests to crop production, especially wheat and barley (Botha et al., 2014; Davis, 2012). The Russian wheat aphid (RWA) *Diuraphis noxia* (Mordvilko), belongs to the family Aphididae, which comprises more than 4300 species specialized to feed on phloem sap (Botha et al., 2014; Douglas, 2006). *D. noxia* populations in South Africa, as well as many other countries, reproduce through facultative parthenogenesis, unlike in areas where it is endemic and can also reproduce sexually (De Jage et al., 2014). The destruction *D. noxia* causes to wheat has resulted in the development of several strategies to control the pest. The primary control mechanism is the use of chemical pesticides followed by biological agents by means of introducing natural enemies, and the use of agronomic practices such as planting dates, early maturing cultivars and crop rotation (Botha et al., 2014; Hajek et al., 2007; Peairs et al., 2006). The efficacy of biological control can be enhanced by coupling it with resistant genotypes, as resistant cultivars exhibit less leaf rolling, which therefore provides predators and parasitoids with easier access to developing aphid colonies (Khan et al., 2013; Jyoti and Michuad, 2005).

Genetic resistance to RWA is also considered a more desirable alternative to the use of expensive, toxic and environmentally hazardous chemicals (Tolmay et al., 2007). Numerous sources of resistance to *D. noxia* have been identified in members of the Triticeae family and are used extensively in the breeding of resistant cultivars (Crespo, 2014; Dogimont et al., 2010). There are several resistant and susceptible wheat varieties available to South African farmers. In South Africa, currently four Russian wheat aphid biotypes have been identified. The first was reported in 1978 and the biotype was designated RWA SA1 (Du Toit and Walters, 1984). In 2005, biotype RWA SA2, virulent against the *Dn*1 resistant gene, was recorded in wheat producing areas, especially in the Eastern Free State (Jankielsohn, 2011; Tolmay et al., 2007). RWA SA3, virulent against the *Dn*4 resistant gene, was recorded in 2009, also predominantly in the Eastern Free State (Jankielsohn, 2011). Recently, RWA virulent against the *Dn*5 resistant gene, designated RWA SA4, has been detected near Bethlehem in the Eastern Free State (Jankielsohn, 2014). Similarly, nearly 30 different strains of leaf and stem rust, and four strains of yellow rust have been identified in South Africa during the past three decades indicating a continued evolution of these pathogens in this country. Oftentimes, the new strains overcome resistance in existing wheat cultivars and hence new resistant cultivars had to be developed to replace the susceptible ones. Thus, the development and application of breeding tools that would significantly shorten the time required for releasing resistant cultivars remains important in South Africa.

Plant response to pathogens and insects

An appropriate response of plants to attack by pathogens and/or insects might result in tolerance or resistance mechanisms that would enable the plant to survive. Therefore, resistance mechanisms are referred to as traits that inhibit or limit infection or insect damage, while tolerance is defined as strategies that do not limit attack but reduce or offset consequences to the plant fitness by adjusting its physiology to buffer the effects of diseases or herbivory (Moreno-García et al., 2014; Lattanzio et al., 2006). Disease tolerance often involves the plant's strategies to compensate for infection damages by increasing the chlorophyll concentration in leaves, increasing the size of new leaves as well as the number of new branches, advancing the timing of bud breaking, delaying the senescence of infected tissues and increasing nutrient uptake (Nabity et al., 2009; Roy and Kirchner, 2000). Resistance strategies on the other hand include physical and/or chemical barriers, mechanisms that rapidly clear infection or deter herbivores such as hypersensitive response (Lattanzio et al., 2006) and processes that limit the spread and damage within the host, such as localized cell death (Lattanzio et al., 2006; Bago et al., 2003).

Plant defence mechanisms

Plants have developed a wide range of defenses against insects and pathogens but these defences do not always protect them against losses in yields (Dangl and Jones, 2001). For pathogens to gain access to nutrients from their host, they must first bridge the natural barriers presented by healthy plants. The first constitutive defence barriers that prevent pathogen entry are the cuticle of epidermal cells and suberized cell walls which contain cutin and suberin respectively (Franke et al., 2012; Freeman and Beattie, 2008). These molecules consist of hydrophobic fatty acid-like polymers that resist biological degradation, except by specialized enzymes (Franke et al., 2012). For haustorium-forming pathogens to cause disease, they must first penetrate the cell wall to establish haustorial feeding structures (Bolton et al., 2008; van Baarlen et al., 2007). However, rust pathogens such as *P. triticina* do not penetrate the epidermis directly but rather enter through the stomatal opening (Bolton et al., 2008). Therefore, rapid closure of the stomata prevents the rust fungus from gaining access to the host plant (Melotto et al., 2008). Papillae (in which secondary antimicrobial metabolites accumulate) deposition at the site of pathogen detection serves as a physical barrier to limit access of pathogens to the protoplast (Clay et al., 2009). Successful halting of the invading pathogen by cell wall-mediated defences at an early stage eliminates the requirement for costlier defence responses such as the hypersensitive reaction (HR) cell death (Moreno-García et al., 2014; Morel and Dangl, 1997). Cuticle and cell wall thickness influence a plant's resistance to certain pathogens by reducing the ability of the pathogen to enter via the thick and tough cell walls (Serrano et al., 2015; Freeman and Beattie, 2008). Thick cuticles physically

prevent the eruption and release of fungal spores; likewise, a waxy cuticle prevents the formation of moisture films on leaf surfaces, inhibiting fungal spore germination (Serrano et al., 2015).

Two major categories of plant chemicals exist in biochemical defences: primary (sugars, proteins, amino acids and nucleic acids) and secondary metabolites (terpenoids, phenols and alkaloids). The primary metabolites are the first substances produced that are important to plant growth and development, while the secondary are involved in plant defence against diseases and insect pests (Freeman and Beattie, 2008; Wittson and Gershenzon, 2002). Plants can also synthesise chemicals such as anti-microbial phytoalexins and saponins that are directly detrimental to pathogens (Bolton et al., 2008; Freeman and Beattie, 2008). Proteins such as protease inhibitors, and lytic enzymes such as chitinases and glucanases, are also produced by the plant before and/or after attack (Doughari, 2015; Ryan and Jagendorf, 1995). Defensive chemicals are toxic to the plant (Wittstock and Gershenzon, 2002), costly in biosynthesis (War et al., 2012), have ecological consequences (Neilson et al., 2013) and are produced mostly after initial damage (Purnington, 2000). Inducible synthesis of defense chemicals is risky, however, initial attack might be too rapid or too severe for the damage-induced defences to be deployed effectively (Wittstock and Gershenzon, 2002). Consequently, the plants that are likely to suffer frequent or serious damage may invest mainly in constitutive defences, whereas those that are rarely attacked rely on induced defences(Dietrich et al., 2005; Koricheva et al., 2004; Wittstock and Gershenzon, 2002).

The exposure of plants to various pathogens or environmental stresses can lead to the activation of inducible defence mechanisms (Rejeb et al., 2014; War et al., 2012; Ton et al., 2009). Induced defence response is dependent on the recognition of the specific pathogen by the plant and its ability to distinguish between different races of the pathogen. The effectiveness of the resistance response is dependent on the rapid recognition of the pathogen-encoded effector protein (avr) by the host resistance (R) gene, a phenomenon known as effector-triggered immunity (ETI) (Harris et al., 2015; Jones and Dangl, 2006). However, if either the plant or the pathogen lacks these corresponding genes, the plant will be susceptible to the infection, as it will be unable to activate defence responses. ETI is especially effective against biotrophic pathogens (Lukasik and Takken, 2009).

Defence responses can occur rapidly through oxidative burst (Ben, 2007; Low and Merida, 1996), localized cell death (Agrios, 2005), accumulation of phytoalexins (Mert-Türk, 2002), synthesis of pathogenesis-related (PR) proteins (Sharma, 2013) and cell wall strengthening proteins (hydroproline-rich glycoproteins) (Torres et al., 2006). They can also enhance transcription of genes, encoding enzymes such as peroxidases, lipoxygenases, superoxide dismutase and phenylalanine ammonia lyase (PAL), involved in the flow of carbon from the primary metabolism into the secondary metabolites (Bolton, 2009; Frost et al., 2008; Tanaka et al., 1989). PAL is a key enzyme in the biosynthesis of phenolic compounds that have antimicrobial activities (Torres et al., 2006; Flors et al., 2005). Delayed defence responses, following further colonization by the pathogen, occur because the plant recognises conserved microbial features such as flagellin, chitin, glycoproteins or lipopolysaccharides (exogenous) generally referred to as pathogen-associated molecular patterns (PAMPs) (Heil, 2009; Jones and Dangl, 2006).

Endogenous plant elicitors are also released following tissue damage, and are referred to as damage-associated molecular patterns (DAMPs) which mediate defence responses to both pathogens and herbivores (Heil, 2009; Jones and Dangl, 2006). Both PAMPs and DAMPs are recognized by plasma membrane-localised recognition receptors (PRRs) (Mazzotta and Kemmerling, 2011; Miya et al., 2007; Huffaker et al., 2006). An immune response triggered by these defence elicitors is known as PAMP-triggered immunity (PTI, previously called basal resistance) with its key component being the hypersensitive response(HR) in the form of localised cell death at the site of pathogen entry (Mazzotta and Kemmerling, 2011; Mur et al., 2008).

ETI is often associated with the accumulation of reactive oxygen species (ROS) and the activation of diverse groups of defence-related genes, including several families of pathogenesis-related (PR) proteins (Mazzotta and Kemmerling, 2011; Ferreira et al., 2007). A few hours to several days after HR development, the un-inoculated portions of the plant often display increased levels of PR gene expression. This leads to the development of systemic acquired resistance (SAR), which is a broad-based and long-lasting resistance to a wide range of pathogens (Mazzotta and Kemmerling, 2011; Boller and Keen, 1999).

Major groups of phenolics in plants

In plants, different phenolic compounds exist with diverse functions and several classes have been categorisedaccording to their basic skeleton as shown in Table 1 (Bhattacharya et al., 2010; Vermerris and Nicholson, 2008).

Phenolics in plant defence

The roles of plant phenolics in defence and communication during Agrobacterium and Rhizobium infection.Although plant phenolics play important roles in plant development, particularly in lignin and pigment

Table 1. Major groups of phenolics in plants and their examples.

No. of carbon atoms	Basic skeleton	No. of phenolic cycle	Class	Examples
6	C_6	1	Simple phenols, Benzoquinones	Catechol, Hydroquinone 2,6-Dimethoxybenzoquinone
7	C_6- C_1	1	Phenolic acids, Phenolic aldehydes	Gallic, salicyclic acids
8	C_6- C_2	1	Acetophenones, Tyrsine derivatives, Phenylacetic acids	3-Acetyl-6-methoxybenzaldehyde, Tyrosol, p-Hydroxyphenylacetic acid, Homogentisic acid
9	C_6- C_3	1	Hydeoxycinnamic acids, Phenylpropenes, Coumarins, Isocoumarins, Chromones	Caffeic, ferulic acids, Myristicin, Eugenol, Umbelliferone, aesculetin, Bergenon, Eugenin
10	C_6- C_4	1	Naphthoquinones	Juglone, Plumbagin
13	C_6- C_1- C_6	2	Xanthonoids	Mangiferin
14	C_6- C_2- C_6	2	Stilbenoids, Anthraquinones	Resveratrol, Emodin
15	C_6- C_3- C_6	2	Chalconoids, Flavonoids, Isoflavonoids, Neoflavonoids	Quercetin, Cyanidin, Genistein
16	C_6- C_4- C_6	2	Halogenated algal phenolic compounds	KaviolA, Colpol
18	$(C_6$- $C_3)_2$	2	Lignans, Neolignans	Pinoresinol, Eusiderin
30	$(C_6$- C_1- $C_6)_2$	4	Biflavonoids	Amentoflavone
Many	$(C_6$- $C_3)_n$, $(C_6)_n$, $(C_6$- C_3- $C_6)_n$	n ≥ 12	Lignins, Catechol melanins, Flavolans (Condensed tannins), Polyphenolic proteins, Polyphenols	Raspberry ellagitannin, Tannic acid

Adapted from Vermerris and Nicholson, 2008.

biosynthesis, they also serve as protective agents, inhibitors, natural animal toxicants and pesticides against herbivores, nematodes, phytophagous insects, and fungal and bacterial pathogens (Lattanzio, 2013; Bhattacharya et al., 2010; Lattanzio et al., 2006, 2008;Dakora and Phillips, 1996). These compounds accumulate in plant tissues and act as phytoalexins, phytoanticipins and nematicides against soil-borne pathogens and phytophagous insects (Lattanzio et al., 2006; Akhtar and Malik, 2000). As a result of these properties, phenolic compounds have long been proposed as useful alternatives to the chemical control of pathogens in crops (Lattanzio et al., 2008; Langcake et al., 1981). In response to pathogen attack, plants accumulate phytoalexins, including hydroxycoumarins and hydroxycinnamate conjugates (Karou et al., 2005). Plants defend themselves against microbial invaders by synthesising, accumulating and releasing the phenolic salicylic acid that plays a central role in many defence strategies (Stewart and Stewart, 2012; Boller and He, 2009). Generally, phenolics are synthesised when plant pattern recognition receptors recognize potential pathogens (Ongena et al., 2007; Tran et al., 2007) through conserved pathogen-associated molecular patterns (PAMPs), leading to PAMP-triggered immunity (Zipfel, 2008) and restricting the pathogen from gaining access to the plant. Phenolic acids are the most common phenolic compounds in cereals and occur as free, bound or conjugated forms. However, most plant phenolic acids are bound by ester-links to the cell polymers (Irakli et al., 2012). In wheat, the main phenolic acids are ferulic and e-coumaric acids, both associated with cell-wall constituents (Okarter et al., 2010). Besides the defensive mechanism of phenols against herbivores and microorganisms, phenolic acids have great potential to improve human health (Navas-Loper et al., 2014). Lignin is a phenolic heteropolymer that plays a central role in plant defence against insects and pathogens (Barakat et al., 2010). It acts by limiting the entry of pathogens by blocking them physically or by increasing the leaf roughness which discourages feeding by herbivores, and also decreases the nutritional content of the leaf (Barakat et al., 2010; Johnson et al., 2009). Herbivory or pathogen attacks have been found to induce lignin synthesis with its rapid deposition, reducing further growth of the pathogen (Johnson et al., 2009). Studies by Barakat et al. (2010) showed an increase in the expression of lignin-associated genes (CAD/CAD-like genes) in resistant plants infected with pests and pathogens.

The oxidation of phenols catalysed by polyphenol oxidase (PPO) and peroxidase (POD) is a potential defence mechanism in plants against insect pests(War

et al., 2012). Quinones are products formed from the oxidation of phenols which bind covalently to leaf proteins and inhibit protein digestion in herbivores (Bhonwong et al., 2009). Further, quinones also exhibit a direct toxicity to insects (Bhonwong et al., 2009; Duffey and Stout, 1996). Another important role of phenols is in the cyclic reduction of reactive oxygen species (ROS) such as superoxide anions and hydroxide radicals, H_2O_2, and singlet oxygen, which in turn triggers a cascade of reactions leading to the activation of defensive enzymes (Maffei et al., 2007).

Flavonoids are another group of phenolics that play a central role in various areas of plant life, especially their interaction in the environment. They also defend plants against various stresses including UV radiation, pathogens and insect pests (War et al., 2012; Treutter, 2006). They are cytotoxic and interact with various enzymes through complexation (War et al., 2012). Flavonoids and isoflavonoids can protect the plant by influencing the behaviour, growth and development of insects (Samanta et al., 2011; Simmonds, 2003). Treutter (2006) stated that flavonoids also scavenge free radicals (including ROS), and reduce their formation by chelating metals. Simmonds et al. (1990) showed that the overexpression of a transcription factor controlling flavonoid production in Arabidopsis, conferred resistance against *Spodoptera frugiperda*.

Tannins are astringent or mouth puckeringly bitter polyphenols that act as feeding deterrents to insect pests, and affect their growth and development by binding to proteins and reducing nutrient absorption efficiency, thereby causing midgut lesions (Stewart and Stewart, 2012; Barbehenn and Constabel, 2011; Sharma et al., 2009; Sharma and Agarwal, 1983). They also play an important role in the resistance of plants against pathogens.

Constitutive and inducible phenols

Plants are known to produce about 8000 types of phenolics, some of which are used as structural materials (lignin), as pigments in flowers, fruits and leaves, as herbivore deterrents (tannins, resins) and in signalling herbivore damage (salicylic acid) (Stewart and Stewart, 2012). Generally, secondary metabolites constitute compounds that do not affect the normal growth and development of a plant, but reduce the palatability of the plant tissue that produces them (Howe and Jander, 2008). Defensive secondary metabolites are either constitutively stored in inactive form in plants or induced in response to insect or microbe attack (War et al., 2012). The former is known as phytoanticipins and the latter is phytoalexins with antimicrobial activity (Ahuja et al., 2012; Gonzalez-Lamothe et al., 2009).

Phytoalexins are isoflavonoids with antibiotic and antifungal properties, and are produced in plants in response to pathogen attacks (Freeman and Beattie, 2008). They are often pathogen- specific in their toxicity and act by disrupting the pathogen's metabolism or cellular structures. Some have been produced by different plants and include camalexin produced by *Arabidopsis thaliana*, medicarpin by alfalfa (*Medicago sativa*), and rishitin by both tomatoes and potatoes (Solanaceae family) (Freeman and Beattie, 2008). Phytoanticipins, on the other hand are found on the plant surface, in vacuoles and organelles as preformed compounds, but they can be released through a hydrolysing enzyme after pathogen attack (Freeman and Beattie, 2008).

Phenolics in response to diseases and their potential as resistance makers

Plant phenols involved in defense are either preformed (constitutive) or synthesised *de novo* (post-infection). Constitutive phenols are mostly antibiotic or antifungal compounds such as simple phenols, phenolic acids, flavonols and dihydrochalcones (Gumul et al., 2007). A plant's defensive response comes from the rapid increase of specific phenolics at the infected site, particularly phytoalexins (Lattanzio et al., 2006; Macheix et al., 2005). These compounds inhibit a broad range of microorganisms, resulting in the development of plant resistance to disease. Polyphenols play role in the resistance mechanism of the plant through their action in programmed cell death of one part of the plant, the rate of which depends on whether the host-pathogen interaction is compatible or incompatible (Lattanzio et al., 2006). It is known that during the establishment of a pathogen in host tissue, there is an increase in the activity of specific enzymes such as PAL, peroxidase and polyphenol oxidase (Lattanzio et al., 2006). These enzymes consume oxygen and produce fungitoxic quinones that make the medium unfavourable for any further development of pathogens. PAL is the key enzyme involved in phenolic compound metabolism through the phenylpropanoid pathway (Dixon et al., 2002). Peroxidase catalyses the condensation of phenol into lignin and is also involved in phenol metabolism (Passardi et al., 2004). Polyphenol oxidase oxidises constitutive plant phenols into quinones, which have bactericidal and fungicidal properties, and is also involved in the oxidation or detoxification of pathogen phytoalexins (Yoruk and Marshall., 2003; Macheix and Fleuriet, 1990). Thus, polyphenols, as well as specific enzymes (PAL, peroxidase and polyphenol oxidase) have proven connections to host resistance to a variety of diseases.

A study by Barbel et al. (1994) on the infection-induced accumulation of phenolic acids in leaves of near-isogenic wheat lines (highly resistant, moderately resistant and fully susceptible to the stem rust fungus) showed that there were no changes in the contents of phenolic acids.

This led to the conclusion that phenolic acids, including cell wall bound cinnamic acids, were not involved in the resistance of wheat to stem rust. However, early studies have ascribed a number of phenolic compounds to the resistant response of wheat to rusts.Flott et al. (1989) reported increased activity of lignin biosynthetic enzymes in a resistant than susceptible wheat cultivar to stem rust. Also in a similar study, host phenol content was associated to resistance to wheat stem rust (Moerschbacher et al., 1989). In other wheat diseases such as leaf rust, take-all, and barley powdery mildew, phenols have been shown to play an important role in disease resistance (Scott-Craig et al., 1995; Rengel et al., 1994; Southerton and Deverall, 1990; Johnson and Lee, 1978).In a soybean-rust pathosystem, Lygin et al. (2009) observed significantly higher cell wall lignification in rust-inoculated resistant soybean lines than in susceptible ones and they concluded that lignin could play an important role in the resistance of soybean to rust.

In sorghums, phenolic compounds such as ferulic acid and tannins are potent inhibitors of pests and pathogens (Chandrashekar and Satyanarayana, 2006). These compounds were found to accumulate mostly in intracellular inclusion bodies, close to the site of fungal penetration, killing both the fungus and the cells that synthesised them (Snyder et al., 1991; Snyder and Nicholson, 1990). It was also observed that the phytoalexin levels reached 150 µM in infected host plants (Snyder et al., 1991). In a similar study on sorghum, phenolic acids, tannins and flavan-4-ols were associated with sorghum grain resistance to fungal invasion (Nicholson and Hammerschmidt, 1992; Jambunathan et al., 1990).

Gogoi et al. (2001) studied the effects of the highly aggressive isolate KB-2 of the Karnal bunt pathogen (*Neovossia indica*) on phenol metabolism, peroxidase (POX) and its isoenzyme on one susceptible and two resistant wheat cultivars. The study revealed that phenols were synthesised at higher than normal levels in resistant genotypes. Three phenolic compounds including caffeic acid, l-tyrosine and hydroquinone, were detected using thin-layer chromatography, while the isoenzymes of POX were detected by polyacrylamide gel electrophoresis (PAGE).Caffeic acid andl-tyrosine were detected at all times at and after inoculation, proving that they can be constitutive or inducible, while hydroquinone was only detected in the resistant cultivar after infection (only inducible).

Abdel-Aal et al. (2001) in another study on wheat, reported that the concentration of ferulic acid (FA), a major phenolic acid of wheat kernel, differs significantly in the mature wheat cultures known to be tolerant to the orange wheat blossom midge (*Sitodiplosis mosellana*). In this study, gas-liquid chromatography (GLC), flurometry, spectroscopy and colorimetry were used to determine the ferulic acid contents of wheat. This method provided a rapid tool in the preliminary screening of experimental lines in the development of resistant wheat cultivars. Similar variation in FA content was observed among barley cultivars (Zupfer et al., 1998) suggesting the potential of FA or total phenolic acids to be used as biochemical markers for disease and insect resistance in wheat and other small grains.

Salari et al. (2013), in a study on the changes of total phenol, total protein and peroxidase activities in melon (*Cucumis melo* L.) cultivars inoculated with *Rhizoctonia solani*, showed that inoculated resistant cultivar roots always had a higher content of total phenol, total protein and peroxidase than their corresponding inoculated susceptible cultivar roots. These and other results clearly indicate that there was a relationship between resistance and accumulation of total phenol, total protein and peroxidase and such information can be utilized in the identification and development of biochemical markers based on phenolics which may be used for rapid wheat rust and RWA resistance screening in South Africa.

CONCLUDING REMARKS

Due to global food security and the consistent increase in the world's population, there is an immediate need to increase wheat yields considerably. Fungal diseases such as wheat rusts and insect pests including Russian wheat aphid (RWA) are on the rise and continue to cause significant losses and pose a challenge to the wheat industry in South Africa. Although genetic resistance provides an effective and environmentally friendly control option, breeding for resistance to rusts and RWA in South Africa involves time consuming and laborious field trials. The identification and application of breeding tools that would improve the rate of cultivar development remains therefore of high priority.Plant breeders have always sought reliable, simple and rapid methods of screening for disease resistance. A broad range of different approaches are now available both to detect resistant genotypes and plants with improved resistances. Among such potentially useful tools are biochemical markers, which are easy to use and can screen large numbers of plants in a short time. The advantage of this technique over phenotypic selection is that it can be performed on infected plants earlier in the infection process, eliminating the expensive and laborious field trials and allowing breeders to precisely and rapidly select resistant germplasm. The present review has clearly shown the presence of a strong association between resistance to wheat diseases such as stem rust, leaf rust, take-all and Karnal bunt and accumulation of phenolics. In addition to wheat, the role of phenols in resistance to pathogens of other hosts like barley, sorghum and soybean has been shown in this review. This information indicates that

phenols present a greater potential of being used as biochemical markers in disease resistance breeding. It is essential for wheat breeding programmes in South Africa to explore this possibility and identify phenol-based markers which may be used for wheat rust and RWA resistance screening, thereby contributing to rapid and sustainable development of resistant cultivars.

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