Chapter 2

Literature Review

2.1. Origin of the air pollutant sulphur dioxide (SO_2) in the atmosphere

SO_2 is one of the major air pollutants that can cause severe damage to vegetation (Malhotra & Hocking, 1977). The molecule is a colourless, corrosive, pungent gas with a molecular weight of 64.06 $g.mol^{-1}$ and is mainly produced by the burning of fossil fuels such as coal, oil and the smelting of mineral ores that all contain a high sulphur content (Gurjar *et al.*, 2010; Chaphekar, 2000; Deepak & Agrawal, 1999). In South Africa, most emissions originate from industrial sources such as power stations situated in the Mpumalanga Highveld region (Mphepya & Held, 1999; Wells *et al.*, 1996). Gurjar *et al.* (2010) stated that SO_2 concentrations of 190.72 ppb should not be exceeded over an average period of 10 minutes, whereas a concentration of 7.63 ppb should not be exceeded for a 24-hour mean period (WHO, 2000). Though these guidelines have been set, annual average SO_2 concentrations of 130 ppb were recorded in 1995 in China’s larger industrial cities, and daily maximum average concentrations exceeded 360 ppb (Yang *et al.*, 2006). Reductions in SO_2 emissions have been recorded since and in 2002 only 22,4% of cities in China reported annual average SO_2 levels exceeding 24 ppb. That being said, even in passing the industrialisation era, the threat that air pollutants pose to other developing countries is far from comforting. Due to the increasing energy demand of developing countries e.g. South Africa, no sustainable sollution is in sight. The detrimental effect of fossil fuel combustion on the natural environment will be an ever worrying issue (Yang *et al.*, 2006; Molina & Gurjar, 2010; Deepak & Agrawal, 2001; Shen *et al.*, 1995, Feng, 2002, Emberson *et al.*, 2001, Yang *et al.*, 2006). The challenge for South Africa will not only be careful monitoring and regulation of emissions, but balancing energy demand, food demand, economic growth due to resource exploitation, import and export of resources, general expectation and quality of life.

2.2. The Highveld region and SO_2 pollution

The term "Highveld" is used to denote the interior plateau of South Africa (Van Tienhoven & Scholes, 2003) and ranges in altitude from 900 to 1800 m above sea level. It is part of the inland plateau of the southern African subcontinent. The Highveld region is situated in a summer rainfall area and includes parts of North-West, the Free State, Mpumalanga and Gauteng provinces (Walker & Schulze, 2008). **Figure 2.1** displays the location of this ecoregion which is bordered by the Drakensberg in the east, the arid Karoo and Kalahari in the west, and the low-lying bushveld to the north (World Wildlife Fund, 2012).

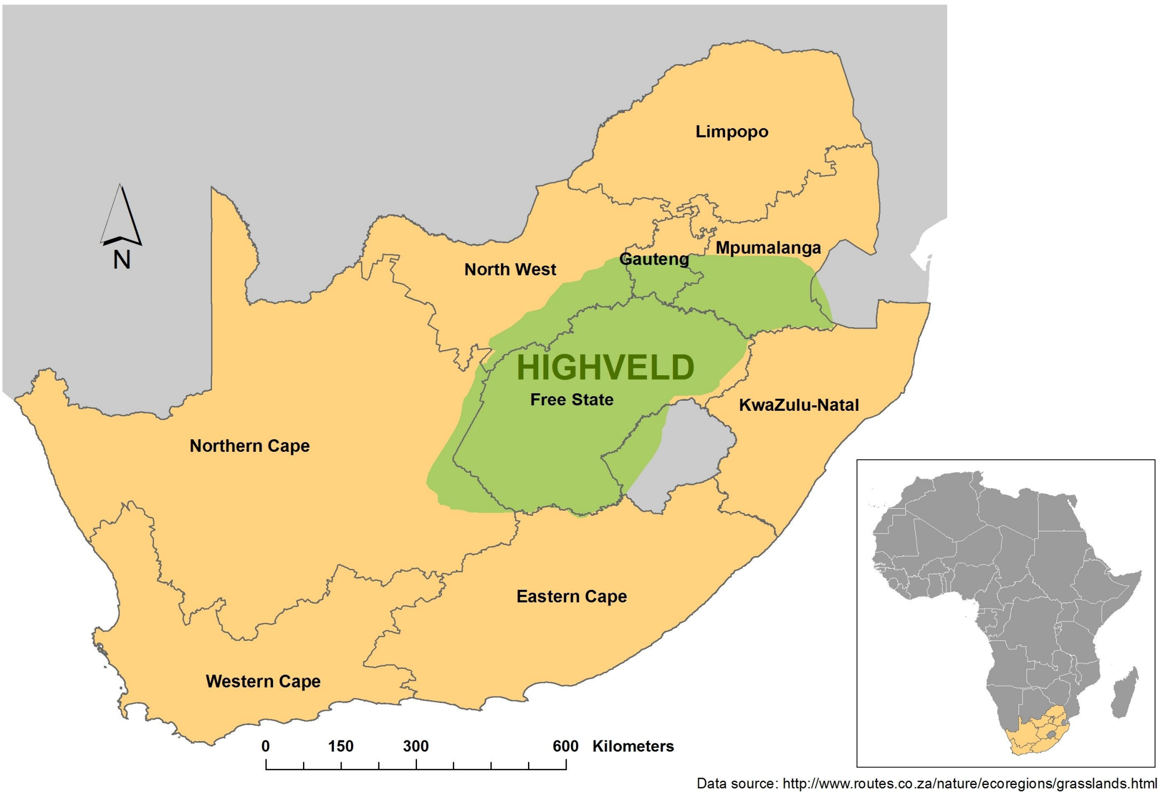

Figure 2.1: Map of South Africa indicating the location of the highveld region (SA Routes, 2005).

South Africa's considerable mineral wealth is exploited by numerous mining and industrial concerns which give rise to more than ninety percent of South Africa's scheduled emissions (Van Tienhoven & Scholes 2003). This often exceeds the critical level (11.5 ppb) for crop yield reduction (Annegarn *et al.*, 1996). Coal extracted from the highveld region in South Africa contributes to 70% of South

Africa's primary energy source. Due to the incredible increase in population size in the past decade, immense pressure is placed on South Africa's non-renewable resources (Ellis, 2001), forcing the over-exploitation of these resources. Over exploitation of coal (**Figure 2.2**) leads to an even further increase in fossil fuel combustion. Consequently a further steep rise in air pollutants due to fossil fuel combustion is expected in South Africa's near future. It needs to be mentioned that apart from the burning of coal in coal-fired powerplants resulting in SO_2 pollution, the extensive mining of coal (and expansion thereof) in the Mpumalanga Province also leads to other forms of environmental pollution (Lawhorn, 2001).

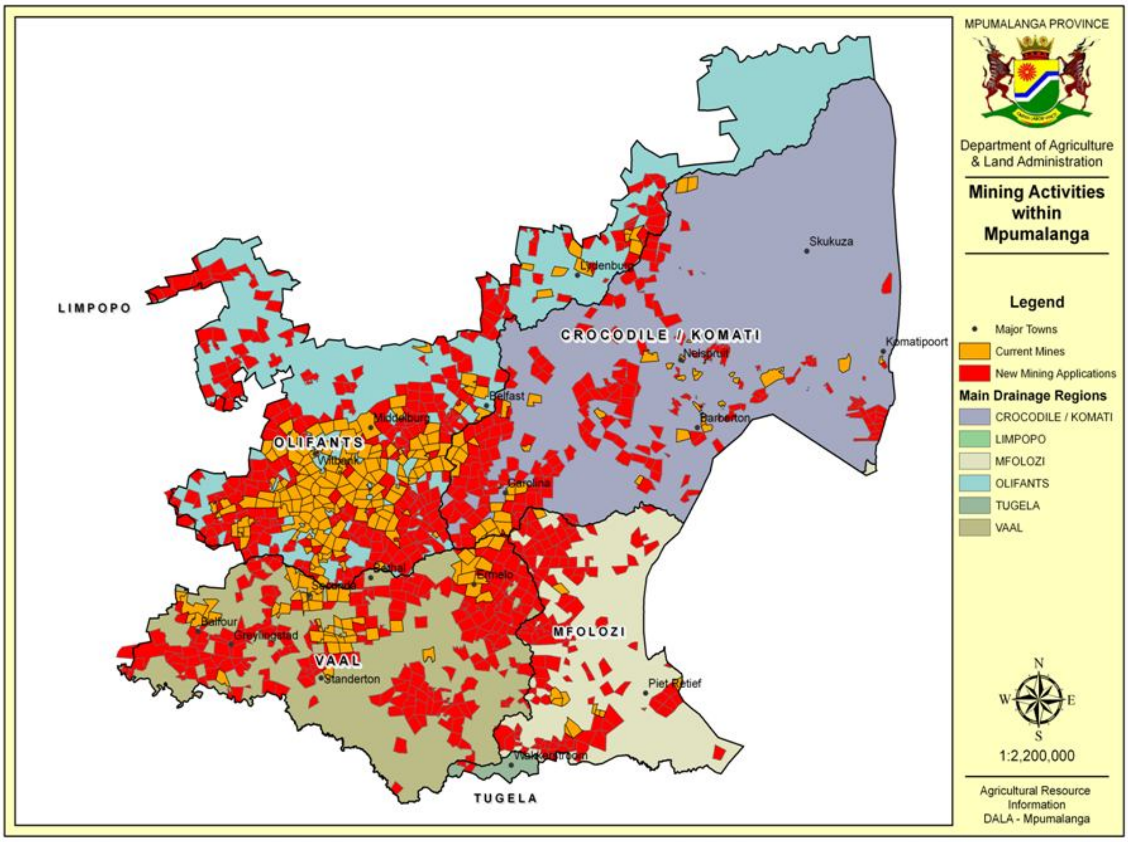

Figure 2.2: The extent of the problem of SO_2 pollution in SA in the foreseen future is illustrated by the expansion of the coal mining industry. The figure illustrates the current (yellow areas) and planned (red areas) coal mines in the Mpumalanga Province. Planned coal mines are represented by those areas for which mining applications have been made (Agricultural Resource Information, 2010).

Prevailing sunny weather that helps to make the region an attractive tourist destination is caused by stable atmospheric conditions that occur for much of the year. However, the stable air layer also serves to trap air pollutants produced during mineral extraction and processing, limiting any vertical distribution, especially in the winter months (Van Tienhoven & Scholes, 2003). Under unstable boundary layer conditions, the plumes can loop down to ground level, with high ground-level concentrations of gases being experienced close to the source (Turner, 1996). The pollutant dispersion conditions within the atmosphere for the highveld region in South Africa have been rated "as among the most unfavourable anywhere in the world" (Tyson *et al.*, 1988). Primary and secondary pollutants can be transported and recirculated over considerable distances within the sub-continent, before exiting over the oceans (Garstang *et al.*, 1996; Tyson, 1998; Van Tienhoven & Scholes 2003).

Agriculture is one of the largest economic sectors in Mpumalanga. The Highveld region of Mpumalanga produces 15% of the total agricultural output in South Africa (South Africa Yearbook, 2003). The growing demand for agricultural products is an important driver of this sector, emphasizing the significance of acceptable land use patterns. The main agricultural crops produced in the Highveld area include sunflower, sorghum, dry beans, soybeans, potatoes, cotton and maize (Jordaan, 2006; Walker & Schulze, 2008). Seventy percent of South Africa's commercially grown cereal crops are cultivated in this region. These agro-ecosystems contribute to ninety percent of maize cultivation in South Africa. Agro-ecosystems in the Highveld region are highly sensitive, and significant environmental impact depends mostly on a changing climate (Union of Concerned Scientists, 2011; Walker & Schulza, 2008). Sustainability is constantly being challenged by the changing climate and the increases in air pollutants. This adds to the vulnerability of commercial maize farmers in the western parts of the Highveld where breakeven yields are just over 2000kg/ha (Du Toit *et al.*, 2000; Walker & Schulze, 2008). Current yields vary between 1000 and 3000 kg/ha, which raise the importance for protocol sustainable practice implementation.

2.3. Effects of sulphur dioxide on plants and its interaction with drought

2.3.1. The effect of sulphur dioxide on plant physiology and biochemistry

The underlying process by which SO_2 induces changes in photosynthesis is difficult to investigate because the photosynthetic rate of a leaf at any time is governed by several physiological factors such as stomatal conductance, biochemical integrity of organelles, membrane integrity, enzyme activities in the leaf mesophyll cells and leaf nutrient content. The link between photosynthesis and plant productivity proves to be a complicated interaction (Winner *et al.*, 1985). Although there have been many investigations on the effects of air pollutants on agricultural crops (Hamid & Jawaid, 2009; Garcia-Huidobro *et al.*, 2001; Kim *et al.*, 2007; Laurence *et al.*, 1981; Lee *et al.*, 1997), studies on the interaction with other environmental factors, particularly under field conditions, are meagre (Verma & Agrawal, 1996).

Vast amounts of literature exist portraying the effects of SO_2, its oxidants and derivatives on vegetation (Swanepoel *et al.*, 2007; Shen *et al.*, 1995; Hamid & Jawaid, 2009; Wellburn, 1987; Yang *et al.*, 2006). Air pollutants have to overcome the cellular barriers present within leaves in order to induce damage. These barriers include the boundary layer, aqueous phase of the apoplast which includes the cell wall and the barrier imposed by the stomata (Luwe *et al.*, 1993; Takahama *et al.*, 1992; Fuhrer, 1996). The toxicity of SO_2 is thought to result from generation of reactive oxygen species (ROS) (Rakwal *et al.*, 2003). The adverse effects of SO_2 and its consequental generation of ROS within the aquous phase inside plant cells, include the destruction of chlorophyll molecules, disruption of the processes of the photosynthetic electron transport chain, inhibition of the carbon reduction cycle (CO_2 fixation) and ultimately a reduction in plant growth and loss in yield (Wellburn, 1987; Heyneke *et al.*, 2012b).

After entering the stomata, SO_2 will combine with the water vapour inside the substomatal space to form sulphuric acid (**Figure 2.3**). The combination of SO_2 with water is the main component of acid rain that affects sensitive ecosystems (Gurjar *et al.*, 2010). Compared to $CO_{2,}$ SO_2 is almost 40 times more soluble in water, causing it to rapidly dissociate in the aquous apoplast to form the very toxic and reactive SO_3^{2-} (sulphite) and HSO_3^- (bisulphite) anions which then enters the cell (Takahama *et al.*, 1992). The ratio in which these anions are formed will depend on the pH of the sollution

(Fuhrer, 1996). The toxicity of SO_2 may either be caused by the negative consequences of acidification of tissues after the dissociation of the absorbed SO_2, and/or the reaction of the formed sulphite with cellular components (Yang *et al.*, 2006; Fuhrer, 1996; Takahama *et al.*, 1992).

$$SO_2 + H_2O \rightleftharpoons H_2SO_3^- \rightleftharpoons HSO_3^- + H^+$$

$$HSO_3^- \rightleftharpoons SO_3^{2-} + H^+$$

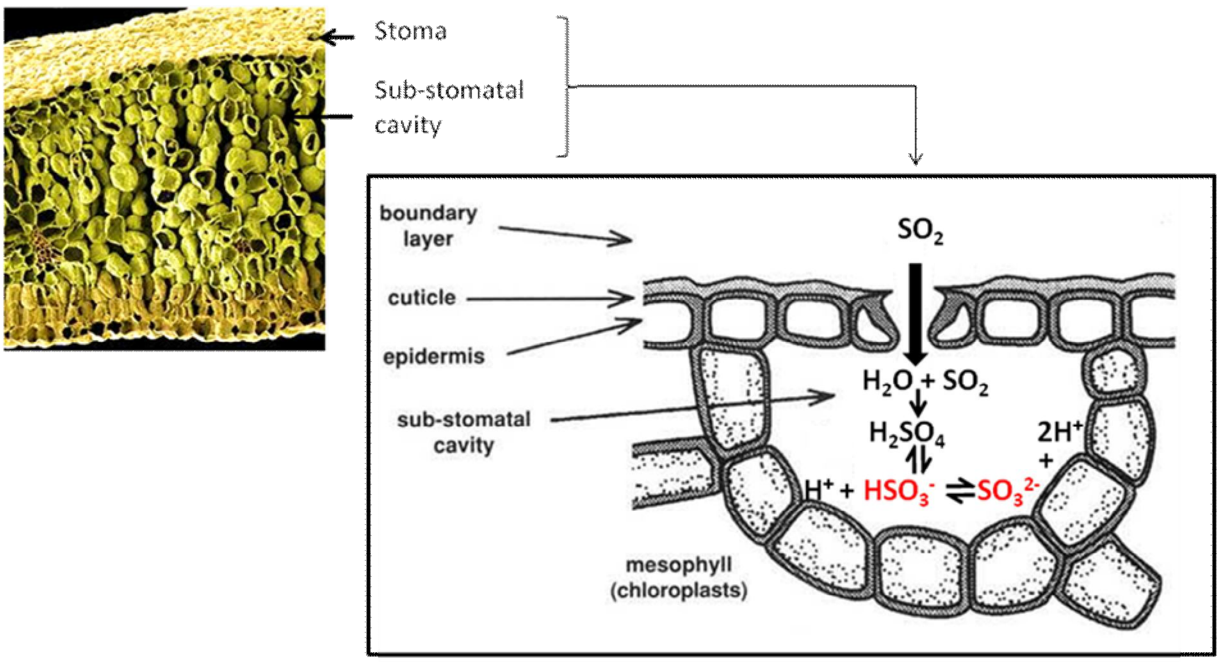

Figure 2.3: The pathway of SO_2 entering the stomata of a C_3 leaf. Also indicating the path after entry, i.e. dissociation of SO_2 in the substomatal air space to form the cytotoxic sulphite (SO_3^{2-}) and bisulphite (HSO_3^-) anions (Schmeissner, 2012; Natural Resources Management and Environment Department, 2012).

2.3.2. Biomass and yield

A number of studies have been carried out to evaluate yield responses to pollution. It has been documented that crop yields declined from 10% to 50% in response to elevated SO_2 concentrations (26.6 ppb – 49.3 ppb) around point sources (Rai *et al.*, 2011; Emberson *et al.*, 2001; Agrawal, 2003). In India crops that were fumigated in open top chambers (OTCs) with 63.5 ppb SO_2 for 70 days, eight hours per day, resulted in yield reductions of up to 25%, and biomass losses of between 4% and 8% (Agrawal, 2003). When SO_2 concentrations were increased even further (149.3 ppb) during accute treatments, yield reductions of up to 57% were recorded. However, research done in China delivered yield-response relationships which indicated that SO_2 gas is the main factor responsible for yield losses, rather than acid rain originating from hydrated SO_2 (Zheng & Shimizu, 2003). Annual crop yield losses due to atmospheric SO_2 pollution and acid deposition damage in eleven provinces of south China, amounted to 4.26 billion RMB Yuan (5.84 billion Rand) (Feng *et al.*, 2002).

2.3.3. Drought as co-stress

The principal climatic driver for agriculture in South Africa is precipitation (Walker & Schulze, 2008). Arid regions occupy about half the surface area of South Africa and experience highly variable rainfall and frequent droughts (Le Houérou, 1996). Natural ecosystems have adapted to this climatic variablity, as have the human societies which inhabit arid zones, demonstrating intricate and diverse adaptation strategies to drought (Seymour & Desmet, 2009). Drought has been researched in South Africa since early last century, and drought losses have long been attributed to poor vegetation, soil and water management (Vogel, 1994).

South Africa will continue to experience droughts, and the likelihood of serious droughts is greater with increasing atmospheric CO_2 levels. Decreases in yield and increased yield variability is quite common with a rise in temperature or reduced precipitation, particularly in the western drier parts of the highveld where rainfall is sometimes the major limiting factor in crop development (Walker & Schulze, 2008). Beadle *et al.* (1993) explains that the reduction in biomass and yield is a result of a reduction the rate of photosynthesis of a crop canopy in response to drought conditions. During water stress conditions the rate of photosynthesis will decline mainly due to stomatal closure and the effects of drought on chloroplast processes. The ability of land users to deal with drought is becoming progressively dictated by the resilience of their agro-ecosystems, the diversity of livelyhood options and whether or not they have access to resources and good institutional support (Vetter, 2009).

What is important to consider, is that plants' reactions to water stress differ significantly at various organisation levels, depending upon the intensity and duration of the stress as well as the plant species and its stage of development (Chaves *et al.*, 2003; Abedi & Pakniyat, 2010). It is therefore imperative for researchers to conduct experiments that include different scenarios with different crops to better equip farmers with predictions and advice according to realistic research phenomena. Unfortunately, research will have no impact on livelihood unless mechanisms are in place for translating findings into policies to address the needs of society in response to drought. The challenges for institutions e.g. the South African Biodiversity Institute and the Agricultural Research Council, is to convey information obtained from research to government, and to convince government for continued research support. The challenge to governement is to translate research recommendations into policy which is extremely difficult, but no less crucial (Seymour & Desmet, 2009).

2.4. Strain, stress and plant responses

2.4.1. Photosynthesis and plant stress

Environmental stresses present major challenges in our quest to achieve sustainable food production. The reactions of crop plants to environmental stresses such as drought and increases in air pollution, are complex and involve various kinds of physiological and biochemical responses (Dubey, 1999; Abedi & Pakniyat, 2010; Kalaji & Guo, 2008). At present, it is very difficult to assess the damage caused by air pollutants (Smith 1947; Woodwell, *et al.*, 1989). Visual injury to foliage is manifested as chlorotic or necrotic lesions on leaves and is often the only availible indicator to evaluate the extent of damage to crops (Heath, 1996). Sublethal plant exposures to a number of phytotoxic air pollutants can cause the suppression of one of life's most vital processes – photosynthesis (Bennett & Hill, 1974).

2.4.2. Photosystem II function and chlorophyll *a* fluorescence induction

Chlorophyll *a* fluorescence is a highly sensitive, non-destructive and reliable tool for measuring the photosynthetic efficiency, particularly of PSII (Stirbet & Govindjee, 2011). PSII has shown to be very sensitive to various environmental stressors (Kalaji & Guo, 2008; Stirbet & Govindjee, 2011; Abedi & Pakniyat, 2010). Grotjohann *et al.* (2004) pointed out that whereas PSI is quite stable in the light, PSII has to pay a high price for its ability to split water, i.e. being sensitive to environmental impacts. Chlorophyll *a* is involved in photon harvesting, in the transfer of excitation energy, in photochemical trapping and in ground state electron transfers. As soon as a green plant containing chlorophyll *a* and the molecules are moved from darkness into light, they start emitting chlorophyll *a* fluorescence. While these photosynthesising plants emit constant fluorescence under steady excitation, the intensity of fluorescence emitted by the photosynthetic organisms changes continuously with time (variable fluorescence), creating characteristic time patterns that are typical of the major taxonomic groups of oxygenic photosynthesisers. These chlorophyll *a* fluorescence time patterns, known also as fluorescence induction patterns, consist of two transients (or phases), a fast one (OJIP) and a slower one, symbolised as SMT (S: slope of initial decrease in fluorescence intensity after P, M: a secondary maximum, T: terminal value). During the O to P phase, fluorescence rises and during P to T, fluorescence declines to a steady state. It is within the fast rise (OJIP) that the activity of PSII is reflected (Papageorgiou & Govindjee, 2011).

2.4.3. Photosynthetic gas exchange

Photosynthesis is the process by which living organisms convert light energy into chemical energy in the form of organic molecules. The importance of photosynthetic organisms as the cornerstone in trophic-hierarchy cannot be emphasised enough, since photosynthesis is the driving force for energy needed in the world (Lawlor, 1993). The most widely used method for assessing the rate of photosynthesis in intact plants is to measure gas exchange. Gas exchange between the leaf and the surrounding air is dependent upon diffusion and is, amongst others, controlled by the opening and closing of stomata. Stomatal movement is extremely sensitive to external environmental factors such as light, CO_2, water status and temperature. An ongoing trade off exists between the uptake of CO_2 for photosynthesis and transpirational water loss. The principal functional advantage offered by the stomatal apparatus is an ability to conserve water by closing the stomatal apperture when CO_2 is not required for photosynthesis or when water stress overrides the leaf's photosynthetic needs. It must be kept in mind, however, that the stomatal opening is not an 'all-or-none' phenomenon. At any given time, the extent of stomatal opening and its impact on both photosynthesis and water loss will be determined by the sum of all factors influencing stomatal function, and not any one alone. It is therefore important to take stomatal function into account when considering photosynthetic productivity and crop yield. Recent interest in stomatal function has been prompted by the recognition that air pollutants such as ozone and sulphur dioxide also enters the leaf through stomata, with a consequent change in stomatal function, photosynthetic capacity and ultimately yield loss can be expected (Hopkins & Hüner, 2004). Drought also inhibits photosynthesis by impairing stomatal function (Yordanov *et al.*, 2003), through the destruction of the photosythetic apparatus, inhibition of the photochemical activities and ultimately decreases the activities of enzymes in the Calvin cyle in photosynthesis. To overcome abiotic stress, plants have consequently devised very impressive defense strategies (Nayyar & Gupta, 2006).

2.4.4. C_3 and C_4 metabolism in plants

Three photosynthetic pathways exist among terrestrial plants: C_3, C_4 and CAM (crassulacean acid metabolism). C_3 and C_4 photosynthetic pathways respond quite differently to changes in the atmospheric environment and are relevant to global change studies. From a global change perspective, the kind of photosynthetic pathway present, influences the magnitude of carbon fixation by the ecosystem, the quality of the plant food resource available to animals and the isotopic composition of CO_2 released to the atmosphere (Ehleringer & Cerling, 2002).

Plants that incorporate carbon solely through the Calvin-Benson cycle (PCR cycle) are generally known as C_3 plants based on the observation that the first product of photosynthesis is a 3-carbon acid, PGA (Ehleringer & Cerling, 2002). **C_3 photosynthesis** is a multi-step process in which the carbon from CO_2 is fixed into stable organic products (**Figure 2.4**). CO_2 is continuously fixed by chloroplasts in the mesophyll layer to a stable, nongaseous molecule 3-phosphoglycerate (PGA). The large majority of global CO_2 assimilation occurs in C_3 plants in which Rubisco (the enzyme that mediates the fixation of CO_2 to the acceptor molecule Ribulose 1,5-bisphosphate) operates at relatively low carboxylation efficiency (Spreitzer & Salvucci, 2002).

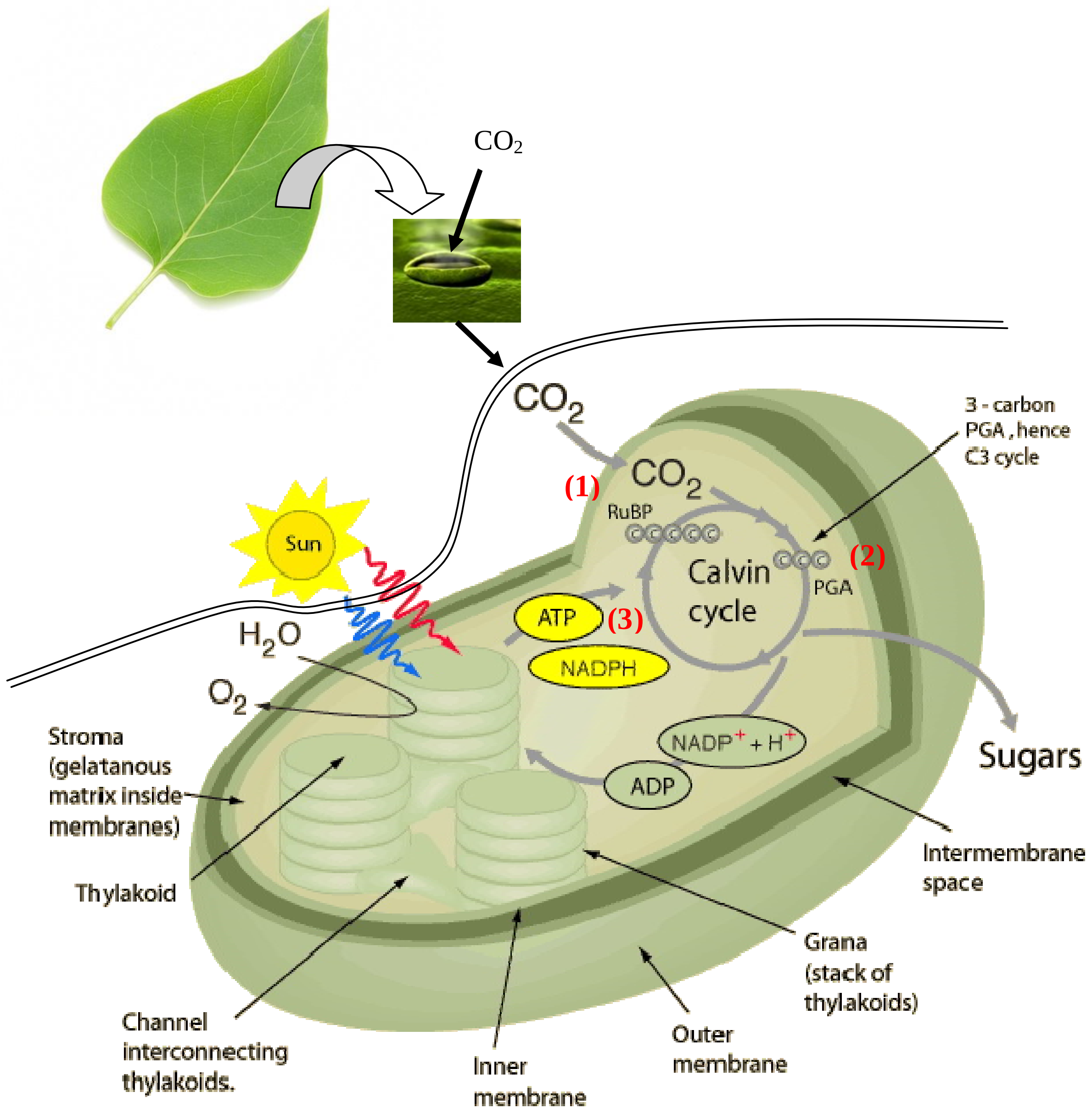

Figure 2.4: The photosynthetic carbon reduction cycle in C_3 plants (Nave, 2012).

This process occurs in virtually all leaf mesophyll cells and can be divided into three primary stages **(Figure 2.4):**

(1) C**arboxylation** fixes CO_2 in the presence of the five-carbon acceptor molecule, ribulose 1,5-bisphosphate (RuBP), and converts it into two molecules of three-carbon acid (3-phospoglycerate). The enzyme that is responsible for the catalisation of the reaction is known as ribulose 1,5- bisphosphate carboxylase-oxygenase (Rubisco) (Sage, 1999). Due to the high concentrations of Rubisco inside the chloroplast stroma, together with the RuBP's high affinity for CO_2, rapid carboxylation at normally low atmospheric concentrations is made possible. This carboxylation reaction yields a six-carbon intermediate which remains bound until it is hydrolysed to two 3-PGA molecules (Ehleringer & Cerling, 2002).

(2) Reduction of 3-PGA, which consumes the ATP and NADPH produced by photosynthetic electron transport to convert the three-carbon acid (3-PGA) to triose phosphate (glyceraldehyde-3-phosphate); and

(3) Regeneration of RuBP, which consumes additional ATP to convert some of the triose phosphate back into RuBP to ensure the capacity for the continuous fixation of CO_2.

Crop plants must have characteristics that make them vigorous, competitive, efficient in the use of available resources, and able to adapt to cultivation conditions. One of the major evolutionary changes that affect these characteristics in plants was the development of the C_4 cycle of CO_2 metabolism (Brown, 1999; Leegoed, 2002). **C_4 photosynthesis** occurs in more advanced plant taxa and is especially common among monocots, such as grasses and sedges and is distinguised from other metabolic pathways by the fact that the initial photosynthetic product is a four-carbon acid, oxaloacetate (OAA) (**Figure 2.5**). In addition C_4 plants exhibit a number of specialised anatomical, physiological and biochemical characteristics (Ehleringer & Cerling, 2002). Photosynthetic rate in C_4 plants may be two to three times higher than that of C_3 plants under conditions of high fluence rates and high temperature (30°C - 40°C) (Leegood, 2002). C_4 plants appear to be better equiped to withstand drought and are able to maintain active photosynthesis under conditions of water stress that would lead to stomatal closure and consequently the reduction of CO_2 uptake by C_3 species.

Such plants possess a CO_2 concentrating capacity, resulting in the suppression of photorespiratory CO_2 loss (Hopkins & Hüner, 2004; Taiz & Zeiger, 1998).

In C_3 plants, the chloroplasts of the mesophyll layer are more or less identical to one another, whereas the chloroplasts of the mesophyll parenchyma of C_4 plants are differentiated into morphologically distinct tissues with different photosynthetic functions: the bundle sheath chloroplasts and the mesophyll chloroplasts. The mesophyll chloroplasts are comparable to those of the C_3 mesophyll chloroplasts, but the bundle sheath chloroplasts are unique in their structure and function in that they serve as compartments where CO_2 is concentrated. In C_4 plants, carboxylation occurs in the mesophyll cell, catalysed by the enzyme PEPcarboxylase (PEPc) that has an exceptional high affinity for HCO_3^-. PEPc consumes bicarbonate ions in reaction with PEP (the acceptor molecule) forming oxaloacetic acid (OAA). In addition PEPc is not inhibited by O_2 (Lawlor, 1987).

$$\text{PEP} + HCO_3^- + H^+ \xrightarrow{\text{PEPc}} \text{OAA} + P_i$$

This C_4 acid, OAA, is then pumped into the adjacent bundle sheath cells where it is decarboxylated to form CO_2 from where it is refixed in the normal C_3 pathway. The PEP reaction acts as an effective CO_2-concentrating mechanism, supplying CO_2 to the photosynthetic carbon reduction (PCR) cycle in the bundle sheath cells. This process continues, even during adverse environmental conditions such as drought and exposure to air pollutants which render the stomatal pores almost closed, and consequently decreasing water loss (Lawlor, 1987; Furbank & Taylor, 1995). The additional cost of C_4 synthesis is the ATP requirement associated with the regeneration of PEP from pyruvate (Ehleringer & Cerling, 2002). No photorespiration occurs in C_4 plants, a phenomenon promoting yield and WUE (Taiz & Zeiger; 1998).

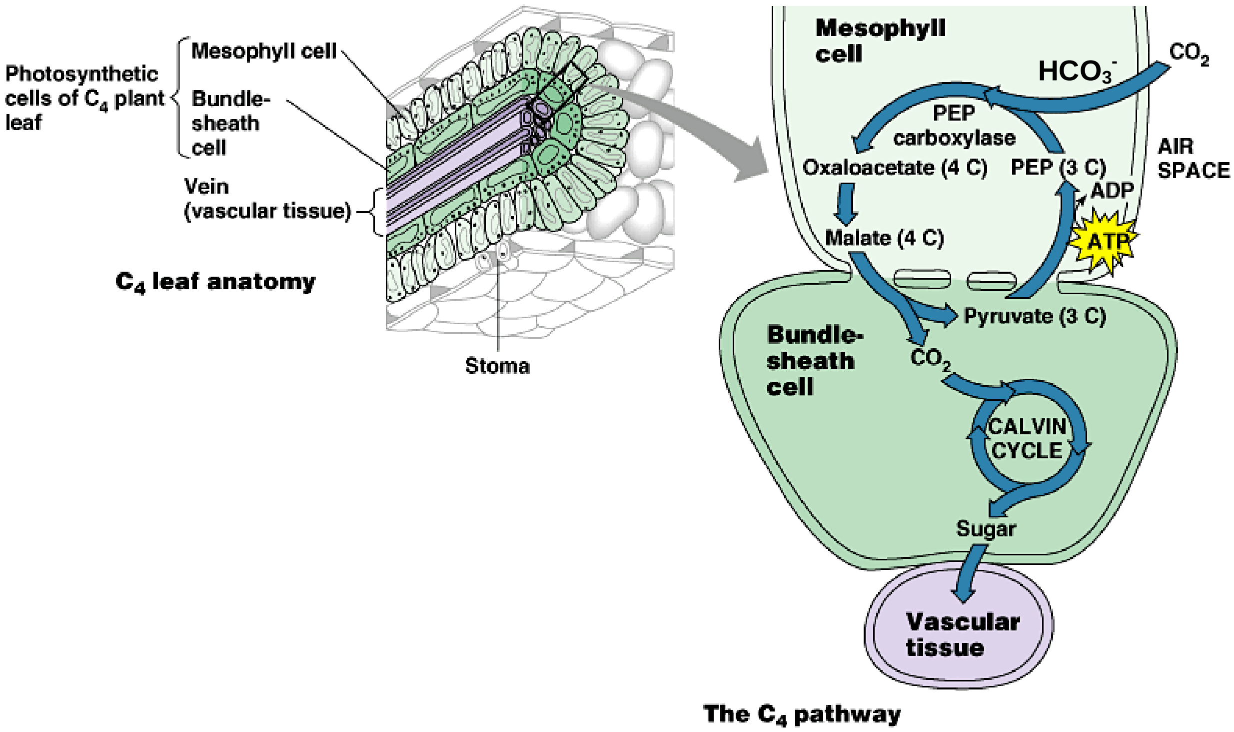

Figure 2.5: The C_4 photosynthetic metabolic pathway (Pbworks, 2011).

2.4.5. Reactive Oxygen Species and detoxification

During their life, plants may be exposed to various biotic and abiotic stress factors such as pathogenic attack, extreme temperatures, air pollutants, drought, excessive light and salinity which often lead to the production and accumulation of ROS. These products comprise of the superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) (Liu *et al.*, 2009; Cruz de Carvalho, 2008; Cheeseman, 2007; Blokhina *et al.*, 2003). Under normal photosynthetic conditions, ROS are produced in the electron transport chains of chloroplasts and mitochondria and as by-products of metabolic pathways in the peroxisome. When ROS are produced in a controlled manner within specific compartments, ROS have key roles in metabolism and molecular biology. These compounds can serve as signalling molecules during pathogenic attack where plants utilise low levels of ROS in signalling pathways that ultimately result in complex, programmed defense responses to the challenger. An example of such signalling is the hyper sensitive response (HR) to pathogens, in which a localised burst of ROS and accumulation of H_2O_2 occur, followed by regions of programmed cell death (PCD) near the infection site (Liu *et al.*, 2009; Apel & Hirt, 2004). If these undesirable cytotoxic by-products however accumulate to excessive levels due to abiotic stress, it can lead to oxidative stress (Gechev *et al.*, 2002). During oxidative stress, singlet oxygen ($^1O_2$) may be produced in the thylakoids which can oxidise membrane lipids, proteins, amino acids, nucleic acids, nucleotides, carbohydrates and thiols. (Melis, 1999; Halliwel & Gutteridge, 1989; Yusuf *et al.*, 2010; Liu *et al.*, 2009; Vranova *et al.*, 2002). The damage caused by these cytotoxic molecules could ultimately lead to the destruction of the photosynthetic apparatus (Noctor & Foyer, 1998) and interfere with CO_2 fixation in the Calvin-Benson cycle. When this metabolic pathway is inhibited, the available pool of $NADP^+$ is depleted which causes the electron transport chain to become highly reduced, leading to further production of ROS and the accumulation of the superoxide ion (O_2^-) in the photosystems. The balance between ROS production and activities of antioxidative enzymes determines whether oxidative signalling and/or damage will occur (Moller *et al.*, 2007; Abedi & Pakniyat, 2010).

To prevent damage, yet allow functions of ROS to continue, antioxidant defenses must keep active oxygen under control (Noctor & Foyer, 1998). The air pollutant SO_2, which may induce ROS in leaf tissue, can be detoxified enzymatically or non-enzymatically during the oxidation of SO_2 to SO_4^{2-}, from where it is mobilised into the vacuole (Tausz *et al.*, 2003). Various antioxidants can be found in the apoplast which include a specific class of peroxidases. These act as signalling molecules during environmental stress and oxidise phenolics using hydrogen peroxide (H_2O_2) as an electron acceptor (Takahama *et al.*, 1992; Heber *et al.*, 1995). Reactive oxygen radicals (O_2^-) can be rendered harmless by the action of the antioxidant enzyme SOD, converting the O_2^- ion to H_2O_2 and O_2:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

The H_2O_2 which resulted from the action of superoxide dismutase (SOD) is also very toxic to cells. The conversion of H_2O_2 to water and oxygen is mediated by the enzymes catalase (CAT) and peroxidase (POD) (Hopkins & Hüner, 2009; Gajehe, 2001). POD is found widespread in nature and is expressed in both prokaryotic and eukaryotic cells. These enzymes can be divided into three classes, namely Class I, II and III peroxidases. The specific class III peroxidases belong to the plant kingdom and are part of the endogenous protective mechanism of plant cells against oxidative stress (Lepedus *et al.*, 2004; Gajehe, 2001).

2.5. Important crops in South Africa

2.5.1. Canola (*Brassica napus*)

Brassica napus is one of the few edible oilseed crops that can be cultivated in temperate agricultural regions of the world, as this crop can withstand low temperatures. *Brassica napus* is commonly grown in Europe and Canada. In south-western parts of South Africa it is a popular winter crop suitable for rotation with wheat (Minnaar, 2012; Kimber & McGregor, 1995; Abedi & Pakniyat, 2010). Canola oil is the third largest source of vegetable oil in the world after soybean oil and palm oil (United States Department of Agriculture, 2012) and the drastic population growth in developing countries is a cause for the increase in demand for oilseed. Growing consumer awareness of the health benefits of replacing saturated oils with monounsaturated and polyunsaturated oils is expected to increase the share canola oil has in world consumption (Nelson *et al.*, 2001).

Although canola is mainly produced for the extraction of edible oil, it is also available for use in livestock diets. Canola seed is high in energy due to its high oil content (40% oil content) (Brand *et al.*, 2001; Kimber & McGregor, 1995). High oil concentration in *Brassica napus* is a requirement for international markets and high protein concentration improves the value of the meal. Oil and protein concentrations are determined by genotype and the environment (Gunasekera *et al.*, 2006). In their study on the effect of different environmental factors and cultivar genotype on oil and protein concentrations in canola and mustard seed, Gunasekera *et al.* (2006) showed that environmental stressors such as increased temperatures and low rainfall, negatively influence the oil concentration of *Brassica napus* seeds. Air pollution is another environmental factor that detrimentally affects *Brassica napus* crops in several ways. Research done by Bosac *et al.* (1993)

demonstrate that the flower buds are the major site of reproductive injury in *Brassica napus* plants, following SO_2 and O_3 exposure.

2.5.2. Maize (*Zea mays*)

Agriculture in southern Africa is important for both export and subsistance purposes. *Zea mays* is widely grown for both commercial and subsistence purposes (Van Tienhoven *et al.*, 2006). Being a C_4 crop implictes that *Zea mays* is adapted to photosynthetically function well under arid, hot, and light-intensive environmental conditions (Schäffner & Sheen, 1992), which is why this crop is viewed as the single most important food crop in Africa (Byerlee, 1996). South Africa alone generates the largest yield quantities in southern Africa (Van Tienhoven *et al.*, 2006), contributing to South Africa’s economical and food security. In eight countries of Eastern and Southern Africa, maize accounts for 50% or more of the calories provided by starchy staples and in many areas, completely dominating the cropping systems of small farmers (Byerlee, 1996).

Although maize production has increased over the last ten years, it has not been sufficient in relation to human population growth (Thottappilly *et al.,* 1993). The average yields for maize in many African countries remain 1-1.5 t/ha which is approximately only a third of the world average (Fajemisin, 2008). Due to the immense increase in population each year, the demand for resources and pressure on the natural environment is increasing. According to estimates, two billion people are suffering from starvation, malnutrition or both. To feed these people and the additional millions to come in the future, all possible methods of increasing the world food supply are currently being pursued (Agrios, 2005).

In South Africa *Zea mays* is widely cultivated, and in the highveld area where SO_2 pollution is an increasing phenomenon, the threat to production is becoming a reality. Though there is evidence of the detrimental effects of air pollution on cereal crops, research on the effect of SO_2 on the physiological or biochemical response of *Zea mays* crops is limited. A risk assessment study of potential agricultural losses due to ambient SO_2 in the central regions of Chile revealed that cereals are affected adversely when exposed to ambient SO_2 concentrations close to large copper smelters. Adverse effects caused by long-term low-level exposures include reduction in shoot growth, yield losses, physiological, chemical and anatomical changes (Garcia-Huidobro *et al.*, 2001). Van

Tienhoven *et al.* (2006) assessed the risk of ozone impacts to *Zea mays* in South Africa. Even though the monitored and modelled data suggest that ozone levels over this region are high enough to potentially damage vegetation, no symptoms or any ozone damage was reporetd for field crops in South Africa. Other environmental constraints on crop production include water shortages. Efleoğlu *et al.* (2009) studied the physiological responses of three maize cultivars to drought stress and found that the growth of all cultivars was retarded under drought stress conditions. In addition PSII function was also impaired. In the present investigation the basis of elevated SO_2 concentrations and drought stress will be quantified and assessed.