1 INTRODUCTION

Brassica napus L. (rapeseed, oilseed rape or canola), an ancient crop plant, belongs to the Cruciferaceae (Brassicaceae) family, also known as the mustard family. Typical oil content of rapeseed ranges from 35-48% DM (NACMA, 1997). Nowadays, it is the third most important source of vegetable oil in the world. Winter rapeseed is a cool-season annual and important oilseed crop which grows in the temperate agriculture zone. In Europe, the total cultivated area covers 7,100,000 ha and the major crop grown is rapeseed with about 4,800,000 ha, the biggest cultivated area of which is in Germany (1,350,000 ha) (Ollier, 2006). The crop is not only used in the food and feed industry, but it also provides a lubricant and a petroleum substitute in bio-diesel. During the 20th century, demand for oilseed rape grew significantly in the developed world (Walker and Booth, 2001). The European harvest in 2005 amounted to 15,500,000 tons, having increased by 1% of the harvest in year 2004 and by more than 28% of the average harvest of the past 5 years (2001 to 2005) (Ollier, 2006).

Since rapeseed is a crop that prefers a cooler climate, the date of sowing will vary according to latitude and date of onset of winter. For example, in Northern Europe optimum sowing date is generally in the latter half of August until the early part of September. Ideally, seeds should be sown into a fine, firm, moist and well-structured seed-bed to encourage rapid and uniform germination and establishment (Almond et al., 1984). Moreover, crop establishment is an important factor to get optimal plant population. Thus, for the highest yield, it is not only the planting time but also the seed rate which affects the plant height, its shape with lodging effect and weed competition (Walker and Booth, 2001). However, the use of rapeseed of low quality leads to a rather poor plant population in the field. In addition, high seed quality plays an important role for production because good seeds contain many components and ultimately achieve final stand of plant. Basra (1995) indicated that high quality seed characterized by having specifically and genetically pure genotype. It should be free from disease, vigorous and high in germination percentage. Thus, the regulation of rapeseed quality is to be recognized as high seed viability and vigour because it supports the rapid germination and fast growth and able to withstand environmental adversity.

During the onset of germination (radicle emergence from the seed) the seed storage reserves (carbohydrate, lipid and protein) are converted into soluble metabolites that can be transported inside the seedling and used to support growth and respiration (Bewley and Black, 1985). Storage lipids located in rapeseeds in the form of triacylglycerols are mobilized and constitute up to 40% of the dry weight of a seed (Slabas et al., 2001). Commonly, during quiescence, after harvesting, seeds have to be stored under various storage conditions. The aging process naturally affects the seed, particularly oil content which is sensitive to deteriorate as a result of oxidation processes. Then, seeds lose their viability to germinate. Moreover, prolonged storage of seeds causes various biochemical processes in addition to moisture and environmental effects.

An understanding of the mechanisms of oil seed deterioration that underline seed qualities and the control of seed metabolism during storage is the objective of this study. Not only lipid biochemical changes were determined but also the products of lipid oxidation, which mainly generate changes in unsaturated fatty acids and affect the structure and functional properties of the cell, were indicated. A major question has been raised how the seed ageing process occurred in various rapeseed cultivars stored under different temperatures for 90 days. Furthermore, this study explored an important progress towards tolerance and protective metabolisms to defend the cell from the attack of reactive oxygen species which include some antioxidant enzyme and non-enzymatic compounds such as tocopherol. The results can not only help to optimize storage conditions before rapeseed cultivation but they are also of importance for oil processing because some stocks may be stored for several months before processing and the oil quality should be maintained. On the other hand, plant breeding programmes can be developed through understanding of rape seed cultivars oxidative stability which could alleviate cell damages. Thus, the seeds can maintain vigour and germinability which are of critical importance for seedling establishment and appropriate plant population.

2 LITERATURE REVIEW

2.1 Seed germination and their dormancy

To be capable to germinate, the seed must be viable and also the embryo alive. Then, with absorption of water and under ambient temperature (20-25°C), growth will be favorable and the seedling will be emerged. The cotyledon of rape seed serves as the primary site of storage lipid and protein metabolism, although mobilization also occurs in the axes (Esau, 1977). Depending on seed maturation time, oilseed synthesizes large amounts of triacylglycerols (TAGs) which are able to completely reverse this process during early germination. During this process the storage oil is rapidly hydrolyzed (Hoppe and Theimer, 1997). With the germination of oilseeds, storage triglycerols are catalyzed by both lypolytic enzymes and enzymes to produce carbohydrates for transportation to the root and shoot axes for the developing of the seedling (Huang et al., 1983). Fatty acids are cleaved by lipase from their glycerol backbone in the oil body and after being transported to glyoxysomes which are organelles found only in stored oil seeds. Then, the fatty acids produced are degraded by β-oxidation to acetyl-CoA which serves as a substrate for the glyoxylate cycle and subsequent gluconeogenesis (Beevers, 1979; Kimberly et al., 1998). Finally, sucrose is the major carbohydrate transport form and is used as a substrate for respiration and biosynthesis of seedling in the storage tissue after seed germination.

Soil temperature is the main factor affecting germination once the seed has imbibed water affecting both speed and proportion to produce a viable plant (Mendham and Salisbury, 1995). Germination time varied with the temperature. It can come as little as one day at 21 to 25°C, but 11-14 days at 2°C (Kondra et al., 1983). For autumn sowings, soil moisture is equally important as temperature.

During germinating time, some seed might not germinate immediately, which were considered as dormant seed and usually exist to delay germination. Oilseed rape has been observed to exhibit little or no primary dormancy (Lutman, 1993; Zhou, 2001). It is believed to be forced into dormancy (secondary/induced dormancy) by abiotic stress conditions such as light, moisture, anoxia or hypoxia and temperature (Pekrun, 1994). The main factors inducing secondary dormancy are low water potentials under dark condition such as might be occur when sowing in a

relatively dry August (Pekrun et al., 1997a, b). Seed storage temperature and duration also influence in secondary seed dormancy expression. This decrease was greater when seed stored at ambient temperatures than stored at -70°C (Gulden et al., 2004). When the seeds overcome the dormancy period, germination begins with imbibition and is finally followed by the emergence of the radicle from the seed coat (Bewley, 1997).

2.2 Processes of deterioration in seeds during storage

2.2.1 Cell structure change

It is generally accepted that the decline in the viability of naturally or artificially aged seed results mainly from damage of nucleic acids and the deterioration of cellular membranes (Osborne, 1982). The ultra-structure in non-aged winter rape seed compared with artificially aged seed was studied by Dawidowicz-Grzegorzewska and Podstolski (1992). In parallel measurements of seed linkages, seed vigour and germinability, it was found that viability of artificially aged seeds was partially or completely impaired and its loss corresponded to an increase in phosphate linkage. Moreover in the age-induced membranes, the coalescence of small storage lipid bodies to larger units was detected, presumably as a result of the degradation of enclosing half-unit membrane. Recently, Walters et al. (2005) studied phase properties of lipids in sunflower seeds. They found that lipid bodies became smaller and more dispersed throughout the cytoplasm during priming and aging.

In addition, the products of lipid peroxidation and associated free radical oxidative stresses affect the structure and function of membranes and includes the inactivation of membrane bound proteins as well as the alteration of membrane permeability (Priestley, 1986; Wilson and McDonald, 1986; Hendry, 1993)

2.2.2 Lipid changes

In general, the storage oils are accumulated in different spherical cell structures called oil bodies (lipid bodies, oleosome) that provide an increased surface area with unique proteins (oleosins) and functional lipid as some phospholipids (Huang, 1992). During storage, aging of the seeds at high temperature and high moisture significantly affects biochemical metabolisms of lipids in the seed. The physical properties of lipids among species during storage under dry condition were compared by using differential scanning calorimetry. This technique measures the

temperature and energy associated with lipid-melting between deteriorated and fresh seed. For all species tested including rapeseed, there was a decrease in the energy associated within the lipid melt in deteriorated samples, and the change occur at a similar rate as the loss of seed vigour. The data suggested that there are changes in lipid components that are associated with seed deterioration (Vertucci, 1992).

Rapeseed lipids contain five major acyl groups: palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2) and α-linolenate (18:3). Typically, rapeseed has the lowest content of saturated fatty acids among vegetable oils and a moderate content of poly-unsaturated fatty acid (Starner et al., 1999). About 90 % of the oil is bound in form of triacylglycerols (Bewley and Black, 1994; Uppstroem, 1995). These major acyl groups are a major constitutes of the cell membrane. With advancing senescence in bean cotyledons (*Phaseolus vulgaris*), there may occur a change in the compositions of phospholipids because the head group and acyl chain of them are susceptible against specific enzymes (Brown et al., 1994). However, some research mentioned this process as lipid peroxidation (Priestly and Leopold, 1979). The generated changes in unsaturated fatty acids affect the structure and functional properties of the cell. These fatty acids are major constituents of all membrane lipids in all parts of oil-containing plants (Fig. 1).

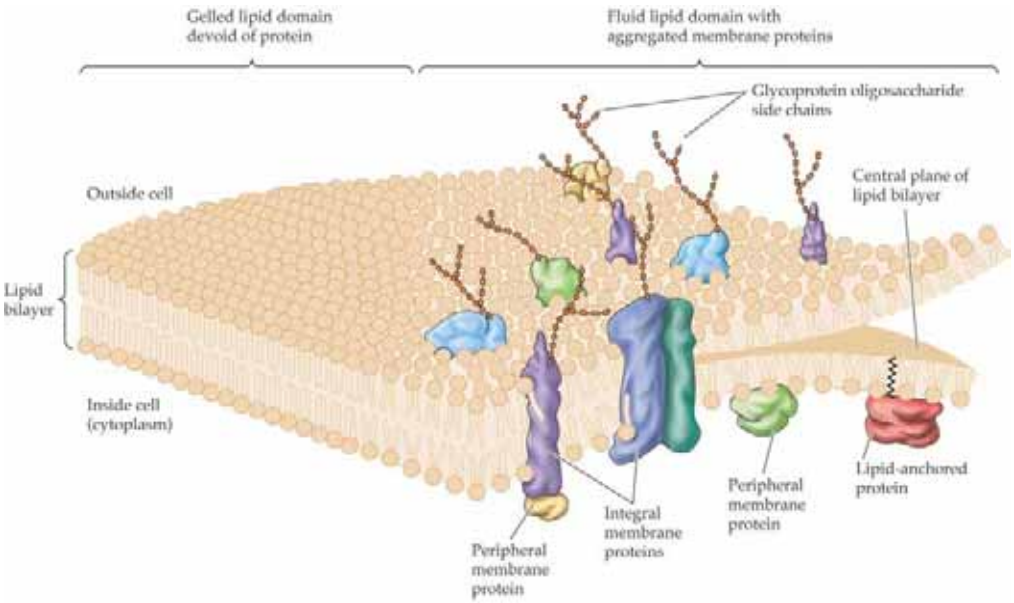

Fig. 1 Structure of plant cell membrane

(www.nrsl.umd.edu/.../Membrane%20Proteins.jpg; Date: 21st December 2006)

Vegetative oil derivatives tend to deteriorate due to hydrolytic and oxidative reactions. These reactions especially hydrolysis of lipids result in the accumulation of free fatty acids (FFA) as well as mono- and diglycerides. Oxidation, auto-oxidation and lipoxygenase induced the presence of high FFA content which will cause later off-flavor in oil seeds (Barker, 1998). The source of primary catalysis which initiate lipid peroxidation are free radicals which contain one or more unpaired electrons (Halliwell and Gutteridge, 1984; Halliwell, 2006), as active forms of oxygen, i.e. the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH°) which can react with biological molecules. In plant cell, many experimental results indicated the production of reactive oxygen species where has been shown in peroxisome (Olsen, 1998; Subramani, 1998; Tabak et al., 1999; del Río et al., 2002, 2006). The major by-product of the peroxisomal metabolism is H_2O_2. The respiratory of mitochondria generally generated through electron leakage to oxygen where under normal physiological conditions ca 1–2% of the oxygen used by the mitochondria is transformed into H_2O_2 (Puntarulo et al., 1988). Moreover, ROS can be enhanced in response to a range of abnormal conditions, including exposure to biotic and abiotic stresses (Rhoads, 2006). The rate of ROS generation in plant cells can bring about extensive oxidative damage (Halliwell and Gutteridge, 2006). These active forms of oxygen may initiate many reactions on polyunsaturated fatty acids leading to the oxidative degradation of lipids or lipid peroxidation which causes various types of cellular damage (Priestly, 1986, Wilson and Mcdonald, 1986; Hendry, 1993; Bailey et al., 1998 and Bailey, 2004). Several comprehensive reviews have identified free radical-mediated lipid peroxidation, enzyme inactivation or protein degradation, disruption of cellular membranes and damage to genetic (nucleic acids) integrity as major causes of seed ageing (Smith and Berjak, 1995; McDonald, 1999).

In soybean, the axes of such aged seeds contain high levels of malondialdehyde (MDA); a product of the peroxidation of unsaturated fatty acids. The levels of linoleic and linolenic acids in a polar lipid (phospholipid) fraction decrease during aging and more dramatically during post-aging deterioration (Robert et al., 1980). Moreover, a significant increase of MDA in the embryo axes and cotyledon of aged peanut seed was identified by Sung and Jeng (1994). Nevertheless, Walters (1998) found that the relationship between the increase of TAGs peroxidation and

decline of viability during aging is rarely clear because decreased un-saturation and increased free fatty acids are usually detected after seeds dies.

2.2.3 Protein changes

Besides mediating lipid peroxidation, O_2^- and derived forms of activated oxygen are capable to oxidize the protein thiol group, causing enzyme deactivation, initiating generation of more reactive and destructive species (Halliwell and Gutteridge, 1984). They generate changes in unsaturated fatty acids that affect the structural and functional properties of a cell resulting in chromosomal aberrations during seed storage (Priestley, 1986; Roberts, 1988). A radical may add to another molecule and seed still has an unpaired electron. The overall effects of lipid peroxidation are to decrease membrane fluidity, make it easier for phospholipids to exchange between the two halves of the bilayer, increase the leakiness of the membrane and damage the membrane proteins, inactivating receptors, enzymes, and ion channels (Halliwell, 2006).

The chemical protein reactions that may lead to the loss of seed viability were investigated by Maillard products. Non-enzymatic reactions, such as Amadori and Maillard reaction could occur even at very low moisture content in seed (Priestly, 1986; Sun and Leopold 1995). This reaction is catalyzed by glycosilation or glycation associated with the covalent attachment of reducing sugars to amines groups of amino acid and proteins to form glycated protein. This results in DNA degradation and impaired transcription which causes incomplete protein synthesis during seed germination (McDonald, 1999). The relevance of the Maillard reaction for the loss of seed viability was investigated by Wettlaufer and Leopold (1991), who observed that seed germination decreased as Maillard products accumulated in soybean embryos. The results suggested the role of non-enzymatic glycation in seed deterioration during accelerated aging. Sun and Leopold (1995) established the correlation between the accumulation of Maillard products and the loss of seed viability under long-term storage conditions. In recent research, Murthy et al. (2002) found that the accumulation of Maillard products in seed axes of *Vigna radiata* Wilczek increased during storage with increasing moisture content and temperature. A decline in seed vigour is also correlated with the accumulation of Maillard products.

2.2.4 Enzyme activity changes

For rape seedling establishment, enzyme activities of the β-oxidation of fatty acids have been identified to occur in both the peroxisomal and mitochondrial cell fractions from cotyledons of rape seedling (Masterson et al., 1990). The most sensitive test for measuring initial seed deterioration is to measure activity of certain enzymes associated with breakdown of food reserves or with biosynthesis of new tissue during germination. Because of the high percentage of unsaturated fatty acids, vegetable oils like rapeseed are susceptible to lipid peroxidation. Many reactions are catalyzed by metals and enzymes, especially lipoxygenase (LOX). The activity of this enzyme correlates with soybean seed deterioration (Narayan et al., 1988a). Lipoxygenase catalyzes the oxidation of polyunsaturated fatty acids by the presence of molecular oxygen. It can be decomposed into acids, ketones, aldehydes or other substances during storage. Alternatively, LOX, present in many unimbibed seeds, is also capable to catalyze the lipid peroxidation by using membrane phospholipid components as substrates (Priestley 1986, Wang et al., 1990). In plants, LOX reacts with the substrate linoleic acid or linolenic acid and generates hydroperoxides. These products are involved in plant defense strategies, wound response, senescence, and development (Hildebrand, 1989). LOX activity is directly affected by storage conditions. Kaukovirta et al. (1998) measured oxidation of linoleic acid in flour suspensions of barley and malt samples. The results showed a great variability of LOX activity depending on the duration of storage. In peanut seed, Sung and Jeng (1994) found that LOX in axis and cotyledon of aging peanut showed a rapid decline in activity after more aging treatment.

2.2.5 Antioxidants and scavenging enzymes on seed aging

2.2.5.1 Enzymatic antioxidants

The ability of seeds to withstand stress might be related to their ability to scavenge active oxygen species in order to avoid among others lipid peroxidation (Leprince et al., 1993; Vertucci and Farrant, 1995) resulting in formation of free oxygen radicals (Wilson and McDonald, 1986). Foyer et al. (1991) indicated that plants have evolved antioxidant systems to protect cellular membranes and organelles from damaging effects of active oxygen species (AOS). Though them cellular damage caused by lipid peroxidation might be prevented or reduced. It

involves protective mechanisms with free radical and peroxide-scavenging enzymes such as superoxide dismutase (SOD, IC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and enzymes of the ascorbate glutathione cycle (Bowler et al., 1992; Foyer et al., 1994). This oxide reduction cycle involves the enzymes ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2), which can react with H_2O_2 and neutralize the activity of AOS (Asada, 1992; Foyer et al., 1991).

Superoxide dismutases are generally considered as key enzymes in the regulation of intracellular concentrations of superoxide radicals and peroxides, these products can react in the Haber-Weiss reaction ($2\,O_2^-\cdot + H_2O_2 \rightarrow OH^- + OH^\cdot + 2O_2$) to form hydroxyl radicals (Bowler et al., 1992), leading to lipid peroxidation (Gutteridge and Halliwell, 1990; Bailey et al., 1996). In addition, APX reduce H_2O_2 to water with ascorbate as electron donor (Asada, 1992). Peroxidase (POD) and CAT are involved in the removal of hydrogen peroxide ($H_2O_2 + AH_2 \rightarrow 2H_2O + A$) (Fridovich, 1986). It is well known that CAT and APX play an important role in preventing oxidative stress by catalyzing the reduction of H_2O_2 (Weckx and Clijsters, 1996). However, it has been shown that seed germinability might be related to the efficiency of free radical scavenging because this preventing may affect seed storability and vigour. In peanut, Jeng and Sung (1994) evaluated the effect of accelerated aging on germinability and several physiological characteristics related to peroxidation in the seed of two cultivars. The results indicated that accelerated aging inhibited seed germination, seedling growth and the activity of SOD, POX, APX and LOX. Baily et al. (1998) has shown that lipid peroxidation resulted in losses of free radical scavenging, which is thought to be involved in deterioration of sunflower seeds during accelerated aging. It was also characterized by a decrease in the activities of CAT and GR. The results suggest that seed germeability might be related to the antioxidant defense systems which play a key role in seed storability and vigour. Investigation about enzymatic changes during storage also indicated that the activity of GR, CAT and APX decreased and were also correlated with the seed vigour. However, the activity of SOD and POX remained unchanged (Murthy et al., 2002).

2.2.5.2 Non-enzymatic antioxidants

Tocopherols or vitamin E are an important class of lipid-soluble compounds with antioxidant activities that are synthesized only by plants and other