GENETIC CHARACTERIZATION OF DORMANCY IN DURUM WHEAT

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Mridull Dilawari

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Mridull Dilawari

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Shahryar Kianian
Chair

Dr. Elias Elias

Dr. Michael Foley

Dr. Philip McClean

Approved:

12/16/2011 — Date

Dr. Richard Horsley — Department Chair

ABSTRACT

Two populations derived by crossing LDN x LDN Dic-3A (Population I) and LDN x LDN Dic-3B (Population II) were genetically characterized for the seed dormancy present on chromosome 3A and 3B of durum wheat. The genes for seed dormancy in these two populations were contributed by the wild parent *T. dicoccoides*. Although the populations showed transgressive segregants for both dormant as well as nondormant parent, the populations were similar to the dormant parent at Langdon and Prosper 2006 field locations for Population I and at Langdon 2007 and Autumn greenhouse season for Population II. Genotypic and phenotypic analysis over the combined populations showed an environmental effect on expression of the trait. Different QTL were identified for both field and greenhouse season for the population derived from the cross between LDN x LDN Dic-3A. Five QTL for seed dormancy were identified on chromosome 3A for the QTL analysis performed over combined field locations. One QTL ranging between marker interval Xcfa2193 and Xcfd2a was consistently present for the 30 day period of seed germination and was also found to be linked to red grain color trait. The QTL analysis performed on the population derived from the cross between LDN x LDN Dic-3B identified only one major QTL on the long arm of chromosome 3B between the marker interval *Xbarc84* and *Xwmc291*. This QTL was consistently present for all the field and spring greenhouse season for the seed germination period of 30 days. The QTL x E effect was also observed for this QTL, however it was very small.

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INTRODUCTION

Wheat is one of the most important crops in the world, with United States (U.S.) being the leading producer followed by Russia, China, Canada, France, Italy, India, Argentina, Australia, and Pakistan (Duke, 1983). Wheat is the basic dietary constituent for a large population in Western Europe, North America, Middle East, West Asia and North Africa. Wheat is mostly used for making various kinds of breads, cookies, cakes, pasta, semolina and beer. Wheat in U.S. is grouped into six classes based on color, hardness and sowing time. North Dakota, is the leading producer of durum wheat with its contribution to total U.S. wheat production averaging 62% for the years 2004 to 2006 (USDA-NASS, 2005; USDA-NASS, 2006; USDA-NASS, 2007). The average domestic consumption of durum wheat as pasta products is 11.8 pounds per capita (Wells and Buzby, 2008). Since, durum is one of the major food ingredients in the diet around the world, it is important to breed for high yield, protein and quality. However, the yield and quality of durum wheat is limited by losses due to climate, diseases and preharvest sprouting. Pre-harvest sprouting (PHS) is a condition where the seeds germinate in the spike even before harvest, in the presence of high temperature and moisture conditions, thereby leading to economic losses due to reduction in yield and grain quality. High temperature coupled with high moisture content leads to some physiological processes such as activation of α-amylase that further cause degradation of starch or carbohydrates in the seed, making sticky crumb and collapsed bread loaves (Kottearachchi et al. 2006). Dick et al. (1980) reported $80 million losses caused due to preharvest sprouting damage of the durum wheat in North Dakota annually. Dormancy is an important trait, reducing damage from preharvest sprouting (PHS). Although, seed dormancy prevents the germination of the grains under high temperature and humidity, this condition should not adversely affect seed germination during planting and the stand establishment when grown in the field.

Dormancy is a quantitative trait, thus, influenced by the environmental factors like temperature, moisture, light, etc. (Nyachiro et al., 2002; Lunn et al., 2002; Reddy et al., 1985; Ueno. K., 2002; Hagemann and Ciha, 1987; Clarke et al., 2005; Osanai et al., 2005) and controlled by many genes (Paterson and Sorrells 1990; Mares 1996; Flintham et al. 2002). In addition to the abiotic factors, physiological hormones such as abscisic acid (ABA) and gibberellic acid (GA) also play an important role in seed dormancy and germination (Nyachiro et al. 2002, Kawakami et al. 1997). Abscisic acid prevents

germination of the seeds while gibberellin induces germination. The environmental and physiological factors interact, thereby making the expression of the trait more complex (Simmons and Sesing, 1990; Holappa and Simmons 1995, Garello and Degivry, 1999).

Dormancy is a polygenic trait. Several QTLs have been identified on all seven chromosome groups in wheat (Anderson et al., 1993; Mares and Mrva, 2001; Kato et al., 2001; Roy et al., 1999; Varshney et al., 2001; Groos et al., 2002). These QTLs were identified in populations derived from red and/or white grain wheat. QTL located on chromosome group 3 have been associated with red pericarp color gene (Rc) (Nilsson Ehle, 1914; Flintham and Gale, 1996). Association has also been observed for dormancy and seed color for other crop species. A transcription factor VIVIPAROUS1 (VP1) located on chromosome 7 of maize, and presumed to control dormancy and grain color, has been identified in wheat on chromosome group 3 (Bailey et al., 1999). VP1 is reported to control dormancy in wheat (Groos et al., 2002; Nakamura and Toyama, 2001). Apart from red pericarp color, seed dormancy has been found to be linked with various other seed traits. In weedy rice dormancy was linked with pericarp color, hull color, seed shattering and brittle rachis traits (Gu et al., 2005). In any event, association between red pericarp color and dormancy has been suggested to be due to pleiotrophy (Flintham, 2000; Warner et al, 2000) or tight linkage between the two traits (DePauw and McCaig, 1983). QTL analysis for dormancy will help identify the physical location of genes responsible for this trait, further leading to cloning of genes for dormancy and incorporation of important loci into present day cultivars by marker assisted selection.

To incorporate dormancy in modern day cultivars, the wild wheat relatives such as *Triticum dicoccoides*, could provide a genetic base to select for genes lost during domestication. Since group 3 chromosomes of *Triticum turgidum* ssp. dicoccoides is reported to have genes for dormancy and red color (Watanabe and Ikebata, 2000), we are targeting chromosome 3A and 3B of the wild relative of tetraploid wheat as a source for dormancy genes. For this reason the objectives are:

1) QTL for dormancy on Langdon- *T. dicoccoides* chromosome 3A [LDN (Dic 3A)] and LDN (Dic 3B) can be identified.

2) Markers flanking the dormancy QTL for future use in marker assisted selection (MAS) can be found.

LITERATURE REVIEW

Durum wheat is one of the most important cereal crops in the world. Preharvest sprouting (PHS) can occur in the presence of high temperature and relative humidity that leads to economic losses due to reduction in yield and grain quality. Dormancy is an important trait that prevents damage from PHS. Although, dormancy prevents the germination of seeds under high temperature and humidity, prolonged dormancy may negatively influence stand establishment under field conditions. Seed dormancy is a quantitative trait influenced by environmental factors and controlled by several genes. Therefore, the literature has been reviewed on various aspects affecting dormancy in wheat.

Dormancy

Dormancy in grains is an important trait that prevents or reduces damage from PHS in the crop. Preharvest sprouting is a condition in which the seeds germinate in the spike before the harvest in the presence of high moisture and temperature conditions. This condition leads to yield and quality losses that further reduce economic gains. Millers and consumers avoid preharvest sprouted wheat grains because degraded starch and proteins, due to increased α-amylase activity, reduces flour thickening power, ultimately reducing pasta quality (Kottearachchi et al, 2006). The mark of good pasta cooking quality is high gluten strength that can be obtained by reducing α-amylase activity.

Dormancy has also been observed in other plant species, such as arabidopsis, rice, maize and barley. The molecular mechanism has been well characterized in arabidopsis (Bentsink et al. 2006, Holdsworth et al. 2008). In wheat, research is under way to understand molecular mechanism of dormancy, gene expression pattern, and regulation. Dormancy is controlled by genetic, physiological and environmental factors. Genes on all 7 chromosome groups of wheat are thought to control dormancy (Kato et al. 2001; Mares and Mrva, 2001; Torada et al., 2005; Imtiaz et al., 2008; Mares et al., 2002; Mori et al., 2005; Sorrells and Anderson, 1995; Lohwasser et al., 2005; Mares et al., 2004; Bailey et al., 1999; Zanetti et al., 2000; Miura et al., 1996; Osa et al., 2003; Kulwal et al., 2004; Flintham and Gale, 1996; Roy et al., 1999; Knox et al., 2005). The physiological processes involving abscisic acid (ABA), gibberellic acid (GA) and cytokinins also regulate the expression of dormancy in wheat (Goldbach and Michael, 1976; Holappa and Simmons, 1995; Suzuki et al., 2000). Genes have been identified that encode for components of these physiological processes (McCarty, 1995; Giraudat et al., 1992; Finkelstein et al.,

2002; Frinkelstein and Lynch, 2000; Brocard-Gifford et al., 2004; Culter et al., 1996; Ghassemian et al., 2000; Nakamura and Toyama, 2001; Nakamura et al., 2007; McKibbin et al., 2002; Johnson et al., 2002; Marchylo et al., 1980, Pessarakli, 2002).

Environmental factors such as temperature, moisture, light, etc. regulate the induction of dormancy (Reddy et al., 1985; Lunn et al., 2002; Nyachiro et al., 2002; Hagemann and Ciha, 1987; Mares, 1983; McCrate, 1981). Different environmental conditions at different stages of seed development control the level of dormancy. Environmental factors also influence the activation of the physiological components that lead to dormancy (Goldbach and Michael, 1976; Holappa and Simmons, 1995). Collectively, the combined action of the genetic, environmental and physiological factors make the understanding seed dormancy a challenging task.

Structure of seed

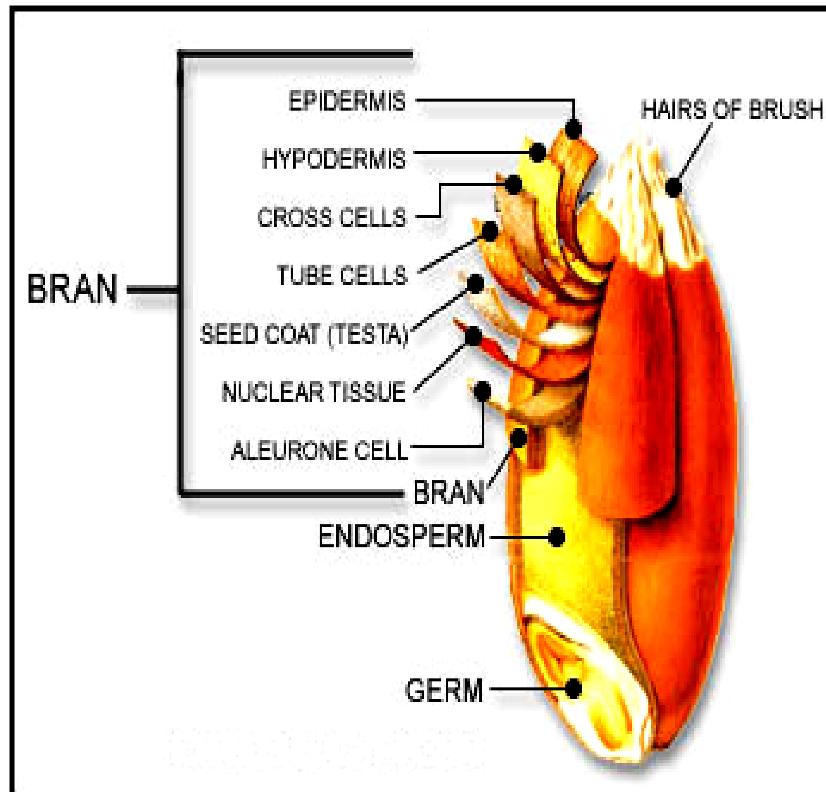
Wheat grain is comprised of three major parts, the bran, the endosperm and the embryo (Figure 1, Hoseney, 1986). Bran is comprised of layers of tissues, like the epidermis, hypodermis, cross cells, tube cells, seed coat or testa, nuclear tissues and aleurone cells.

Pericarp (Fruit coat): It is the outer most surface of the seed, maternally derived from the ovary wall and is composed of several layers of tissue. Outer pericarp is comprised of epidermis, hypodermis and remnants of thin walled cells. Removal of these layer leads to movement of water into the pericarp (Hoseney, 1986). The inner pericarp is made up of intermediate cells, cross cells and tube cells. The total pericarp comprises about 5% of the total grain (Hoseney, 1986).

Seed coat (Testa): The seed coat is between the tube cells and the nuclear tissue. It is comprised of thick outer cuticle, a pigment layer and the thin inner cuticle. Pigment layer is absent in the white wheat. This layer is also maternally derived from the ovule coverings or integuments (Hoseney, 1986).

Nuclear tissue: It is the thinnest layer between the seed coat and the aleurone layer and is about 7μm in thickness (Hoseney, 1986).

Aleurone layer: This layer surrounds both the endosperm and the germ and is comprised of the aleurone cells (Figure 1). Aleurone layer is thinner over the embryo because of the absence of these cells. It is rich in thiamine, riboflavin and has high enzyme activity. It forms the part of the bran and is usually removed while milling. The enzymes present in the aleurone layer are responsible for the degradation of starch in



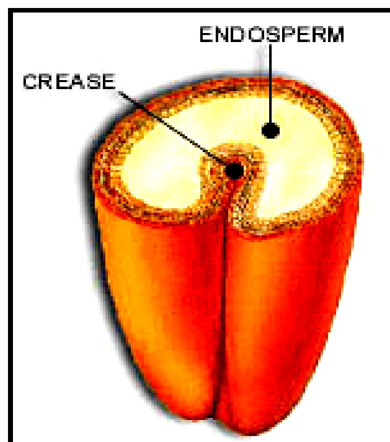


Figure 1. Structure of wheat grain (Hoseney, 1986).

the endosperm during the germination process (Hoseney, 1986).

Embryo: It is the diploid sporophytic tissue formed as a result of the fusion of the egg nucleus with one of the sperm nuclei (Gelin, 2002). It is composed of the embryonic axis i.e., the rudimentary root and the shoot, and the scutellum that acts as the storage organ. It comprises of 2.5 to 3.5% of the wheat grain. It is rich in enzymes and a good source of vitamin B and vitamin E (Hoseney, 1986).

Endosperm: This is the starchy part of the seed and the food reserve for the embryo. It is triploid tissue derived by fusion of one sperm nucleus (male) with two polar nuclei (female) (Sargant, 1900; Dumas and Mogensen, 1993; Russell, 1993). It is composed of the peripheral, prismatic and central cells. Its cell walls are composed of pentoses, hemicelluloses and the □-glucans and the thicker the cell wall the more water absorption. For that reason, the hard wheats have higher water absorption than the soft wheats (Hoseney, 1986).

Germination process

Imbibition of grain leads to the activation of GA in the embryo, which leads to transcription of □-amylase genes and transcription of □-amylase, and starts degradation of the starch and proteins into sugars and amino acids, respectively in the endosperm that is further absorbed by scutellum and transferred to the embryo for its growth and development into the root and the shoot (Belderok, 1968). There are two types of □-amylases produced by wheat grain known as □-Amy1 and □-Amy2 (Gale and Ainsworth, 1984). The production of □-Amy1 and □-Amy2 is controlled by GA and ABA (Marchylo et al., 1980). ABA is known to prevent the activity of the □-amylase thereby, preventing germination of the seeds leading to dormancy. Genes responsible for the regulation of □-amylase enzyme have been mapped on chromosome groups 6 and 7 (Nishikawa and Nobuhara, 1971). Other than ABA and GA, cytokinins are also reported to influence the germination of seeds. Cytokinins are responsible for cell division and its interaction with ABA prevents the blockage of the GA stimulation (Pessarakli, 2002). Suppression of cytokinin activity will lead to embryo dormancy (Pessarakli, 2002).

Types of dormancy

Taiz and Zeiger (2002) suggested that there are two types of seed dormancy that have been recognized. Coat imposed dormancy is caused by the presence of tough seed coat or other related tissues like glumes, lemma, palea, pericarp and endosperm covering the embryo. These non-permeable

layers of tissues prevent the access of water and oxygen to the embryo, as a result, it fails to germinate. In addition, sometimes the seed coat and the endosperm also produce inhibitors like ABA that lead to dormancy. Presence of lignified and waxy layer can also lead to coat imposed dormancy.

Embryo dormancy is an inherent conditions of the embryo. High ABA content and reduced levels of GA content in the embryo lead to embryo dormancy. Different genetic components have been shown to control dormancy imposed by either seed coat or embryo (Flintham, 2000). White wheat was shown to have different degrees of dormancy in the presence or absence of introgressed red grain color (R) alleles in the white wheat background. The presence of this variation was attributed to the genetic component other than the R allele that is presumed to control seed color and dormancy traits. PHS genes are believed to be the genetic component that led to the variation in the dormancy, even in the presence of the R allele. PHS is supposed to control embryo imposed dormancy, however, R alleles are found to control the seed coat imposed dormancy as well (Flintham , 2000).

Seed dormancy can also be classified as primary and secondary dormancy depending upon the time of occurrence of dormancy. If the seeds are dormant when released from the plant, it is called primary dormancy. However, if the seeds produced were afterripened or nondormant and become dormant after being subjected to specific environmental conditions, it is called secondary dormancy (Taiz and Zeiger, 2002). Secondary dormancy is not well understood as a result of environmental effects.

Genetics of dormancy

Dormancy is a quantitative trait and thus influenced by many genes and/or environment (Miura et al., 1996). In the population of 66 recombinant chromosomal lines for chromosome 3A developed by crossing Chinese Spring (CS) and CS (Timstein 3A) substitution line, the frequency distribution pattern revealed a discontinuous and bimodal distribution for percent germination (Miura et al., 1996). Due to the presence of variation within the lines and the transgressive segregants with 0% germination it was hard to estimate the number of genes responsible for the bimodal distribution pattern. However, high level of transgression could only be explained by presence of more than one gene responsible for the trait.

Many genes have been identified for dormancy and PHS located on different chromosomes of wheat. Major QTL for dormancy were identified on chromosome group 3 of wheat (Bailey et al., 1999; Zanetti et al., 2000; Imtiaz et al., 2008; Mares et al., 2002; Miura et al., 1996; Osa et al., 2003; Mori et al.,

2005; Kulwal et al., 2004; Lohwasser et al., 2005; Flintham and Gale, 1996). The ones located on chromosome group 3 were mostly associated with red seed coat color (Flintham and Gale, 1996) and could be attributed to coat imposed dormancy. While analyzing the populations of 120 and 160 recombinant inbred lines (RILs) each carrying the single gene for redness contributed by one of the parent, derived from a cross between red grained 'Red bobs' with white grained 'Kenya 321', red grained 'Dollar' with Kenya 321 and red grained 'Spica' with CS, Flintham and Gale (1996) observed the association of the dormancy trait with the red grain color trait. They identified that Red Bobs, Dollar and Spica contributed R-A1, R-B1 and R-D1, respectively. The degree of dormancy was enhanced in white grained wheat NS67, after incorporating red color genes from any one of the five different donors. Thereby, indicating red seed color is associated with dormancy trait**.** In addition to this, they also identified the homologous relationship for dormancy genes in different cereal crops. Molecular markers from wheat and barley were used to generate the map. As a result they identified the distal region of the long arm of chromosome group 3 in wheat and 3H in barley, where the red color gene maps is highly conserved and paralogous. Similar results were also observed by Imtiaz et al (2008). They studied a mapping population of 271 BC1F7 individuals derived from a cross between a red grained dormant 'Syn37' and white grained non-dormant, 'Janz' to identify QTL for dormancy. Dormancy was assessed using germination index (GI), visible sprouting (VI) and sprouting index (SI). All the three assessment criteria showed significant correlations, thereby indicating that either same or closely linked genes are controlling the expression of the dormancy trait. Single locus analysis identified two QTL on chromosome 3D using GI and VI assessment criteria. However, only one QTL was detected using SI selection criteria. QTL *Qphs.dpiv-3D.1* detected on chromosome 3D explained 37%, 15% and 7% of variation for the trait using GI, VI and SI measures of dormancy, respectively and was found to be associated with red seed coat color trait. Another QTL identified on chromosome 3D, *Qphs.dpiv-3D.2* explained 11% and 3% of phenotypic variation for the trait using GI and VI measures of dormancy.

QTL for dormancy identified on chromosome group 3 is not always associated with the red color gene located on the same chromosome group (Bailey et al., 1999). A QTL was identified on chromosome group 3 that was not linked to the red grain color. This QTL was the orthologue for the transcription factor viviparous-1(VP1) present on maize chromosome 3 and is responsible for grain color and other spike

related characters in maize, and is also present on the long arms of wheat chromosomes 3A, 3B and 3D and rice chromosome 1 (osVP1). All the three loci in wheat mapped 30cM from centromere and 30cM proximal to R loci that controlled seed coat color and coat imposed dormancy. Thus, absence of tight linkage between R loci and VP1 could provide a great potential for developing PHS tolerant lines without red grain color. Similar results were also observed by Zanetti et al. (2000), when they identified a QTL for dormancy present on chromosome 3B and not associated with red grain color trait. In another study by Miura et al., (1996), the results were in accordance with the ones observed by Zanetti et al., (2000) and Bailey et al., (1999). They identified genes for seed dormancy to be present on chromosome group 3 of CS, however, this QTL was not associated with the red grain color trait. To support their hypothesis, they evaluated 24 substitution lines for group 3 chromosomes, in which the group 3 chromosome was substituted from 8 varieties of diverse origin into the CS background. The germination experiments revealed that genes for dormancy were present on all the three group 3 chromosomes. The chromosome 3A from 'Timstein' when incorporated into the CS background showed significant increase in the level of dormancy. However, no association could be found between red grain color gene and QTL identified for seed dormancy trait. Chromosome 3B contributed by varieties 'Cheyenne', 'Lutescens 62' and 'Synthetic' into CS background, also showed increase in dormancy but were not associated with the red grain color trait.

Identification of a QTL is also dependent on the developmental stage of the evaluation. Given QTL may show varied effect at different developmental stages of the plant. In a mapping population of 125 RILs derived from a cross between red grained, highly dormant 'Zen' and red grained, moderately dormant CS, Osa et al. (2003) identified two major QTL for dormancy on chromosome 3A of wheat using 19 marker loci to generate the map of 250cM. One major putative QTL was identified on the terminal region of the short arm of chromosome 3A and was designated as *Qphs.ocs-3A.1* and explained 23.3 to 38.2% of phenotypic variation for the trait. However, the effect of this QTL was small at the dormancy breaking stage. *Qphs.ocs-3A.2* was identified at the dormancy breaking stage on the long arm of chromosome 3A and explained only 13% of variation for the trait. Osa et al. (2003) did not find the two dormancy QTL to be associated with the red seed coat color trait. *Qphs.ocs-3A.2* was linked with *tavp1*, the orthologue in wheat for VIVIPAROUS-1 (*Vp1*), the transcriptional factor related to dormancy in maize

(Bailey et al., 1999), by 46.6 cM, thereby suggesting that effect of *Qphs.ocs-3A.2* was not attributed to *tavp1* or R-A1 locus. Both QTL were contributed by the Zen allele. *Qphs.ocs-3A.1* was also identified by Mori et al. (2005) in another study and explained 11.6 to 44.8% of phenotypic variation for the trait.

Introgression of QTL for dormancy into the white wheat could lead to higher levels of dormancy (Kottearachchi et al., 2006). In a population derived from a cross between red grained Zen and white grained Spica. Kottearachchi et al. (2006) studied the effect on dormancy when QTL *Qphs.ocs-3A.1*, identified by Osa et al. (2003) and Mori et al. (2005), introgressed into the white wheat background. As a result, they identified that some of the white grained lines with introgressed Zen allele for dormancy at *Qphs.ocs-3A.1* locus showed higher level of dormancy with germination index of 0.25 to 0.4, compared to the red grained lines with Spica allele for dormancy at *Qphs.ocs-3A.1* locus with 0.5 to 0.6 germination index in glasshouse experiments.

QTL, environment and epistatic interactions make the expression of the dormancy trait even more complex. Kulwal et al. (2004), performed QTL analysis for PHS on the 110 RILs of the ITMI population derived from a cross between 'W7984', a synthetic wheat and 'Opata85', a bread wheat. As a result using single locus analysis five QTL were identified on chromosome 2BL, 2DS, 3BL and 3DL. Three QTL had alleles for PHS contributed by the less dormant parent. The phenotypic variations for the trait were explained by these QTL individually, ranged from 8.12 to 17.39%. Two locus analyses identified a total of 14 QTL that comprised of main effect QTL (M-QTL), epistatic QTL (E-QTL) and QTL identified with QTL x environment (QE) and QTL x QTL x environment (QQE) interactions. Out of 14, eight M-QTL spanning chromosomes 2B, 3B, 3D and 6A and accounting for 47.95% of variation for the trait were identified. Eight E-QTL spanning chromosome 2B, 3B, 3D and 5B were also identified, explaining 28.73% of phenotypic variation for the trait. Of these eight E-QTL, five overlapped with M-QTL. Five QTL located on chromosome 2B, 3B, 6A and 7B showed QE and QQE interaction that accounted for 3.24% of phenotypic variation for the trait. Two QTL, located on chromosome 3BL and 3DL each, were significant QTL, as they were identified using both single and two locus approaches in more than one environment, above the threshold LOD score of 3.63, 5.48 and 4.70 for environments II, III and IV, respectively. The QTL identified on chromosome 3DL showed the LOD score of 4.16 and 5.60 in environment II and III, respectively, while the one identified on chromosome 3BL had a LOD score of 4.72 in environment IV.

These two QTL could be used in the marker assisted selection (MAS) to breed for PHS resistance. In another study by Mori et al. (2005), the QTLx E interaction was observed in a population derived from a cross between Zen and CS. Seed dormancy was estimated by germinating seeds at 15^0C and 20^0C. A QTL, identified on chromosome 4BL between marker loci *Xgwm495* and *Xgwm375*, explained 11 to 19.8% of phenotypic variation for the trait. This QTL was present only at the germination temperature of 20^0C, while absent at 15^0C. Thereby, indicating that different environmental conditions, like in this case difference in temperature during seed germination, could also have an effect on the QTL for dormancy.

Putative QTL for dormancy showing major effect have also been identified on chromosome 4 of wheat (Kato et al. 2001; Mares and Mrva, 2001; Torada et al., 2005; Imtiaz et al., 2008; Mares et al., 2002; Mori et al., 2005; Sorrells and Anderson, 1995; Lohwasser et al., 2005; Mares et al., 2004). Kato et al. (2001) identified three QTL for dormancy located on chromosome 4 of *T. aestivum* using 119 double haploid lines derived from a cross between 'AC domain' and 'Haruyutaka'. One major QTL mapped on long arm of chromosome 4A and two minor QTL that mapped on long arm of chromosome 4B and 4D showed homology. These three QTL together explained 80% of total phenotypic variation for seed dormancy trait. The increased seed dormancy alleles were contributed by AC domain. Comparative mapping between wheat, barley and rice showed homoeologous relationship between QTL located on chromosome 4AL of wheat and SD4 gene controlling seed dormancy, located on chromosome 4H of barley. However no homoeology was observed with chromosome group 3 in rice carrying major QTL for seed dormancy. Similar results were also observed by Mare and Mrva (2001). In a double haploid (DH) population derived from a cross between an extremely non dormant line, 'Cranbrook' and a moderately dormant line, 'Halberd', they identified a QTL for dormancy on chromosome 4AL of wheat. However, this QTL had a minor effect for the dormancy trait. Torada et al. (2005), however, identified a major QTL on long arm of chromosome 4A explaining 43.3% of total phenotypic variation for the trait via SSR using 96 DH derived from a cross between PHS resistant variety 'Kitamoe' and a non resistant line 'Munstertaler' (K/M). This QTL was consistently present in another population of 96 DH lines derived from 'Haruyutaka'/ 'Leader' (HT/L) and 67 BC1F2 plants from 'OS21-5'/ 'Haruyokoi' (O/HK) and explained 28.5 and 39% of total phenotypic variation for the trait under greenhouse condition. However, no significant effects were observed under field conditions. Thereby indicating that the above QTL cannot be used for marker

assisted selection (MAS), as it was not showing consistent effect in all environment. In another study Imtiaz et al (2008) identified two major QTL, designated as *Qphs.dpiv-4A.1* and *Qphs.dpiv-4A.2* on chromosome 4A using GI, VI and SI, as dormancy assessment criteria. *Qphs.dpiv-4A.1* explained 25, 17 and 11% of variation for the trait using GI, VI and SI, respectively as measures of dormancy, While *Qphs.dpiv-4A.2* explained 23, 24 and 12% of variation for the trait, respectively. Mori et al. (2005) also identified QTL for dormancy on long arm of chromosome 4A of wheat between the marker loci *Xcdo795* and *Xbcd808*, explaining 13.6% of phenotypic variation for the trait. Mares et al. (2004), in a population of 181 double haploid lines derived from a cross between non-dormant, 'Cunningham' and a dormant Chinese spring line 'SW95-50213', identified a QTL for dormancy on chromosome 4A explaining 11-13% of variation for the trait. Two markers flanking this QTL were *Xgwm397* and *Xwmc468* and were 8cM and 12cM from the putative QTL, respectively. The QTL was found to be contributed by the dormant parent in this population.

Different genetic components could be controlling dormancy and PHS traits in wheat (Lohwasser et al., 2005). Using the introgression lines developed by Pestsova et al. (2001) for *Aegilops tauschii*, Lohwasser et al., (2005) studied the influence of the D genome on PHS and dormancy in wheat. As a result, only one major QTL for dormancy was identified on chromosome 6DL and a minor QTL on short arm of chromosome 6D. However, no QTL was identified for PHS. The QTL was contributed by the *Aegilops* parent for non dormancy.

In other studies, many minor QTL have been observed on chromosome groups 1, 2, 5, 6 and 7 (Roy et al., 1999; Knox et al., 2005). Minor QTL for PHS were identified by Roy et al., (1999), in a population of 100 RILs derived from a cross between red colored, PHS tolerant genotype, 'SPR8198' and white colored, PHS susceptible 'HD2329' genotype. They identified two markers linked to PHS tolerance. The STMS (sequence-tagged microsatellite site) primer pair for the locus wmc104 and the STS (sequence- tagged site) primer pair for the locus *MST101* showed linkage with the trait and were found to be present on chromosome 6B and 7D, respectively. The results also indicated that tolerance to PHS in 'SPR8198' is governed by two genes exhibiting complementary gene interaction. Similarly, Knox et al. (2005), also identified a minor QTL for dormancy on chromosome 1A of wheat. They performed the genetic analysis of PHS on the population of 98 RILs derived from a cross between two durum wheat

lines, a non dormant line ‘CI13102’ and a moderately dormant line ‘Kyle’. Dormancy was measured as germination resistance index. Different lines were showing trangressive segregation in different environments thus indicating interaction of environment with the expression of the trait. Heritability for the trait over three environment was 60%. Simple interval mapping (SIM) revealed one QTL located on chromosome 1A.

Minor QTL for dormancy were also identified on chromosome group 2 of wheat (Mares and Mrva, 2001; Mares et al., 2002). Researchers identified QTL associated with grain dormancy in Australian wheat. Two QTL explaining 11 to 9% of phenotypic variation for the trait and located on chromosome 2AL and 2DL, were identified. The alleles for these QTL were contributed by the non-dormant parent. Imtiaz et al, (2008) also identified three minor QTL on chromosomes 1D, 2D and 6D using mixed linear model. However, the effect of these minor QTL was not significant, so the QTL identified on these chromosomes could not be used for developing the cultivars with resistance to PHS. In another study Sorrells and Anderson (1995), in a population of 78 RILs derived from a cross between ‘NY6432-18’ and ‘Clark Cream’ (CC) identified six QTL for dormancy present on different chromosomes. Locus *Xbcd1434* located on 1AS, *Xcdo431* on 1BS, *Xcdo64* on 2S, *Xcdo795* on 4L, *Xbcd1874* on 5L and *XksuG12* on 6L all showed association with this trait. Three major QTLs, associated with marker loci *Xbcd1434*, *Xcdo431* and *Xcdo64* together explained 32% of variation for the trait, using multiple-regression model. The former two were contributed by ‘CC’ allele and led to reduced sprouting, however, the later was contributed by ‘NY18’ allele and resulted in greater resistance to PHS. High heritability was observed for the trait. Homology was also observed for these QTLs among wheat, barley and maize.

Association of dormancy with other traits

Dormancy is associated with various spike related characters in different crops. Gu et al. (2005) identified dormancy to be associated with the hull color, seed coat color, and seed shattering traits in weedy rice. In wheat it has been associated with the red seed coat color trait. Metzger and Silbaugh (1970) performed monosomic analysis to identify the location of the genes for red seed coat color in *Triticum aestivum* using the F2 and the backcross populations. As a result they identified three genes for the seed color trait to be located on the chromosome group 3 of wheat.

The QTL for dormancy associated with red grain color have also been identified on the same chromosome group by Groos et al. (2002). QTL analysis on 194 RILs derived from the cross between 'Renan' and 'Recital' was performed to study the relationship between red grain color and PHS. 'Renan' has red grain color and is resistant to PHS while 'Recital' is white grain and is susceptible to PHS. Using SSR, RFLP and AFLP, QTL for PHS red grain color were detected. As a result, four QTL identified for PHS were also present for red grain color. Three of them were present on the long arm of group 3 chromosome, close to previously mapped R gene and tavp1 loci. The allele for resistance was contributed by Renan. One QTL for PHS and grain color was detected on long arm of chromosome 5A and the resistance allele was contributed by Recital.

Similar results were also observed by Watanabe and Ikebata (2000). They analyzed two RIL populations derived from a cross between amber grained Langdon (LDN) and red grained Langdon dicoccoides 3A (LDN Dic- 3A) and a cross between LDN and Langdon dicoccoides 3B substitution lines (LDN Dic-3B) for dormancy. As a result they observed that the red grained lines showed reduced germination in comparison to the amber grained lines. Thereby, indicating that dormancy is associated with the grain color. Chromosome 3A showed higher level of dormancy than chromosome 3B. In another study by Imtiaz et al (2008), QTL for dormancy, designated as *Qphs.dpiv-3D.1*, explaining 29 to 43% of variation for the trait was also associated with the red grain color trait.

The QTL for PHS resistance was also found to be associated with the ear morphology trait in wheat (Zanetti et al., 2000). Researchers performed genetic analysis of PHS resistance in wheat and spelt cross using 226 F5 RILs derived from a cross between Swiss wheat cultivar 'Forno' and Swiss spelt cultivar 'Oberkulmer'. PHS was measured by estimating falling number (FN) and alpha amylase activity (AA). QTL were identified using 183 loci mapped on 204 RILs. As a result 12 and 13 QTL were detected for FN and AA, respectively, explaining 75% of phenotypic variation for the trait. The two traits were found to be highly correlated and had 9 QTL in common between them. Three of the six QTL with major effects on PHS resistance coincided with QTL for ear length. The QTL with strongest effect had the positive allele from Oberkulmer and was located on chromosome 5AL at the q locus that is responsible for the ear morphology. QTL located on chromosome 6A had positive allele contributed by 'Forno' and QTL present

on chromosome 3B and 7B had positive alleles from Oberkulmer improved PHS resistance without changing ear morphology.

Influence of environmental factors on dormancy

Dormancy is a quantitative trait thus influenced by several environmental conditions like temperature, moisture, light, etc. Variation in degree of dormancy is the function of genotype, environment and genotype x environment interaction.

Different temperature conditions at different stages of plant growth affect seed dormancy. The effect of temperature on seed dormancy was studied in five winter wheat cultivars by Reddy et al. (1985). Three of these cultivars produced white soft grains, one of them produced amber red grains and another one had hard red grains. The plant material was tested for dormancy by germinating seeds at 15, 20 and 26^{0}C for the material produced at 15^{0}C, 26^{0}C and field temperature conditions at grain development stage. Researchers identified two of the white wheat lines to be more non dormant than the rest of the three lines. They also observed that dormancy was found to be higher in all the five lines when the temperatures were lower at the grain filling stage. However, as the temperature increased from 15 to 26^{0}C during the grain filling stage, there was considerable loss in seed dormancy. Loss of dormancy was also observed, when the seed germination temperature was below 15^{0}C. However, significant increase in dormancy was observed when the seed germination temperature increased from 15 to 26^{0}C. The effect of field conditions on seed dormancy was same as that observed at 26^{0}C because the average temperature in field was 20-26^{0}C. Thus, implying that temperature affects dormancy at various plant growth stages like grain filling stage, at seed maturation and germination stage. Higher temperatures during seed development stage and lower temperatures at seed germination are responsible for breaking seed dormancy (Reddy et al., 1985). Thereby, indicating development of lines that are dormant at high temperatures during grain filling stage and resistant at low temperatures during pre harvest stage is needed to prevent pre harvest sprouting damage as a result of cooler and humid climate.

The effect of temperature on seed dormancy during different seed developmental stages was also observed by growing experimental material under different environmental conditions for multiple years (Lunn et al., 2002). As a result they identified that lower levels of dormancy was observed when the mean temperature in the field during seed development stage was high. Similar results were also

observed by Nyachiro et al. (2002) when they found that with the increase in the germination temperature from 10 to 30C, the level of dormancy also increased. They also reported that high amount of variation for dormancy was observed in the population at 15 and 20C.

Effect of temperature is also observed at the after-ripening stage. After-ripening is the process undergone by the seeds before they germinate. Some seeds show high level of dormancy and thereby require after-ripening so as to break dormancy. However, length and temperature of after-ripening affects the degree of dormancy. After-ripening is also dependent on the genotype. Some genotypes require longer after-ripening period than others. Flintham and Gale (1996) subjected plant material to germination test after 1, 2 and 3 weeks of after-harvest-ripeness. They observed that the rate of germination was dependent on the genotype, days of dry storage and the time within each test. It was also observed that dormancy diminished faster in seeds after-ripened for longer time. The effect of different temperatures immediately after harvest and during after-ripening on seed dormancy in three winter wheat lines grown in five different environmental conditions, was studied by Hagemann and Ciha (1987). 'Moro' was soft white club wheat, 'Nugaines' was soft white non club wheat and 'Wanser' was hard red common wheat. Germination tests were performed to study dormancy at 15 and 30^{0}C immediately after harvest and after 8 weeks of dry storage at -10, 10, 20 and 30^{0}C. As a result researchers identified that, low level of dormancy was observed at low germination temperatures of 15^{0}C. As the temperature was increased to 30^{0}C the level of dormancy also increased. Loss in seed dormancy was observed in seeds after-ripened for 8 weeks at higher temperatures of 30^{0}C. High level of dormancy was observed by seeds stored for 8 weeks at -10^{0}C in comparison to the ones stored at higher temperatures. Moro otherwise a non dormant cultivar, showed high level of seed dormancy when stored at -10C for 8 weeks. Thereby, indicating that temperatures below zero preserve seed dormancy and after-ripening process at higher temperatures break dormancy. Similar results were also observed by Mares (1983) when they found that seed dormancy was conserved at low temperatures of -15^{0}C, provided the grain moisture content was less than 12-15%.

In addition to the temperatures at seed development stage, seed germination stage and at after-ripening stage, there are other factors that influence degree of seed dormancy. These can be the type of genotype and other environment factors like moisture/humidity during different stages of plant

development that could affect seed dormancy. Variations in seed dormancy were also attributed to loss of water soluble growth inhibitors present in the seed, as a result of leaching during seed imbibitions (McCrate, 1981).

Effect of ABA on dormancy

The environmental conditions and their effect on various physiological factors like ABA content, presence of which increases seed dormancy, makes detection of dormancy even more complicated. ABA is the key factor for dormancy, present in embryo of wheat and many other crops like Arabidopsis, rice and barley. Goldbach and Michael (1976) attributed the effect of temperature on the level of ABA content in seeds and to seed dormancy. Researchers observed that the seeds ripened at higher temperature of 26^0C, lost ABA much faster than the seeds developed at 18^0C. Thereby, indicating that higher temperatures led to degradation of ABA in the seeds during ripening process.

The effect of dehydration, cold temperature treatment and osmotic stress on a protein PKABA1 (abscisic acid (ABA)-induced protein kinase) that is supposed to control ABA level in seeds was observed by Holappa and Simmons (1995). They observed that within one hour of dehydration the level of ABA and protein PKABA1 increased 500 fold. The level of ABA and PKABA1 was high for 24 hours and declined thereafter. Similarly low temperature treatments led to increase in ABA, PKABA1 and 3 LEA (late embryogenesis abundant) mRNA levels in the seeds after first 24 hours of cold treatment. High level of ABA was observed in scutellum, shoot and root tissues. Scutellar tissues showed higher level of ABA in comparison to shoot and root tissues. However, level of ABA decreased after 3 days of cold treatment. PKABA1 was also found to be upregulated by salt and osmoticum treatment.

The level of ABA present in the seed varies at different stages of development (Suzuki et al., 2000). Suzuki et al. (2000) observed that level of ABA is the maximum from 30 to 45 days after pollination (DAP) in the seeds derived from the dormant source, thereby, indicating a high level of ABA present in the embryos from the period of middle to late embryogenesis. The level of ABA tends to reduce in the embryos obtained after 30 and 40 DAP and when subjected to incubation at 20^0C. This was also associated with increased germination of these embryos. Thus, indicating that level of ABA decreases with time thereby leading to the loss of embryo dormancy.

Besides the environmental factors governing the level of ABA content in the seeds, there are genetic factors that are involved in controlling the level of ABA mediated dormancy in seeds at different stages of seed development. Extensive work has been done in Arabidopsis to identify the genes involved in ABA signal transduction pathway. About 6 ABI (ABA insensitivity) and 2 ERA (enhanced response to ABA) genes have been identified in Arabidopsis (Finkelstein et al., 2002), along with their homologues in different crop species. ABI3, orthologus to maize VP1 gene (McCarty, 1995), is a B3 type transcription factor and responsible for seed dormancy (Giraudat et al. 1992). ABI5 is a bZIP transcription factor, is responsible for regulation of seed maturation, germination (Frinkelstein and Lynch, 2000) and retardation of shoot growth (Lopez-Molina et al. 2002; Bensmihen et al. 2004). ABI8 is involved in germination inhibition (Brocard-Gifford et al., 2004). ERA1 encodes beta-subunit of protein farnesyl transferase (Culter et al. 1996) and ERA3/ EIN2 encodes for a membrane protein similar to Nramp family of metal ion transporters (Ghassemian et al. 2000) and also involved in ethylene signaling pathway. It is responsible for hypersensitivity to ABA during seed germination and high levels of seed dormancy. Homologues to these genes have been identified in wheat, for example, TaVP1 and TaABF are the homologues of ABI3 (Nakamura and Toyama, 2001; McKibbin et al. 2002) and ABI5 (Johnson et al. 2002), respectively.

Homologues for the genes involved in ABA signal transduction pathway in Arabidopsis and QTL for dormancy were identified by Nakamura et al. (2007), in a population of 115 RILs derived from a cross between *T. monococcum* (Tm) and *T. boeticum* (Tb). They identified four homologues, TmABI8, TmERA1, TmABF and Tm VP1 on chromosome $3A^m$. The gene order was similar between the rice homologues on chromosome 1 and wheat homologues on chromosome $3A^mL$, thereby indicating synteny between the two crop species. TmERA3 was located on chromosome $5A^m$. An orthologues for barley SD1 and SD2 was identified on chromosome $5A^m$ and chromosome $4A^m$ of wheat, respectively. The homologue of SD1 was marker ABC302 and that of SD2 was 5E15 and 5E16.

The QTL analysis revealed four QTL for seed dormancy (Nakamura et al., 2007). The major QTL for dormancy was located on chromosome $5A^mL$ of wheat between marker loci *Xcdo1326c* and *XABC302* and explained 20-27% of phenotypic variation for the trait. This QTL could be the orhtologue for barley seed dormancy QTL, SD1. Three minor QTL, two on chromosome $3A^mL$ of wheat, in regions TmABI8 and TmABF and one on chromosome $4A^m$ at locus *Xrz141*, together explaining 10% of phenotypic variation

for the trait, were detected. All the four QTL detected were contributed by Tb allele to increase seed dormancy. The two QTL identified on chromosome $3A^m$ were co-segregating with TmABF and TmABI8, thus could be the possible candidate genes for seed dormancy. Two SNPs were also identified between Tb and TmABF, thereby making the possibility of TmABF to be the candidate gene for dormancy even stronger.

Although many QTL have been identified on different chromosomes of wheat, however, not all identified QTLs are suitable for breeding because they do not show consistent high effect and are not constantly present in different environmental conditions. Group 3 chromosomes are thought to have genes for dormancy and also contain the red grain color genes (Watanabe and Ikebata, 2000). Since chromosome 3A and 3B of *Triticum dicoccoides* are believed to be involved in dormancy (Watanabe and Ikebata, 2000) the present research was undertaken with the main objectives to:

1) Identify the QTL for dormancy on Langdon - *T. dicoccoides* chromosome 3A [LDN (Dic 3A)] and LDN (Dic 3B).

2) Identify markers flanking the dormancy QTL, for future use in marker assisted selection (MAS).

Therefore hypothesizes being tested by this study are:

1) Hypothesis for 3A

Ho: No gene(s) for dormancy exists on chromosome 3A in Langdon derived from *T. dicoccoides* Israel A (Joppa, 1993).

If the hypothesis is proven incorrect then the following is tested.

Ho: No markers flanking the gene(s) can be identified.

2) Hypothesis for 3B

Ho: No gene(s) for dormancy exists on chromosome 3B in Langdon derived from *T. dicoccoides* Israel A (Joppa, 1993).

If the hypothesis is proven incorrect then the following is tested.

Ho: No markers flanking the gene(s) can be identified.

MATERIALS AND METHODS

Plant material

The experimental material comprised of Population I with 83 recombinant inbred chromosomal lines (RICL) developed by crossing 'Langdon -16' durum *T. dicoccoides* chromosome 3A substitution line (LDN (Dic- 3A)) with Langdon-16 (LDN-16) and Population II with 91 RICL obtained by crossing LDN (Dic-3B) with LDN-16 (Joppa, 1993). Both LDN (Dic-3A) and LDN (Dic-3B) were the dormant parents and LDN-16 was the non dormant parent. Several popular durum cultivars such as ‘Alkabo’ (Elias and Manthey, 2007), ‘Grenora’ (Elias and Manthey, 2007), ‘Divide’ (Elias and Manthey, 2007), ‘Dilse’ (Elias et al., 2004), ‘Belzer’ (Elias et al., 1999), ‘Plaza’ (Elias et al., 2001b), ‘Lebsock’ (Elias et al. 2001a), ‘Chahba88//B’, ‘Iact12/Ka’, ‘Montrail’ (Elias and Miller, 2000b), ‘Maier’ (Elias and Miller, 2000a), ‘RI4137’, ‘Pierce’ (Elias et al., 2004) and ‘Ben’ (Elias and Miller, 1998) were used as checks in Population I, while Ben, Lebsock, Montrail, Divide, Chahba88//B, RI4137 and Iact12/Ka were used as checks in Population II. These populations were used to identify the QTL for dormancy present on chromosome 3A and 3B of *T. dicoccoides*. Choice of material is due to previous studies indicating that chromosome 3A and 3B have significant effect on dormancy (Watanabe and Ikebata, 2000).

The plant material for Population I was grown in greenhouse in Fall 2004 for seed increase. The seeds thus obtained were further used in Spring 2005 greenhouse to test for dormancy trait and also for seed increase. Since the number of seeds obtained from the spring season was less, the plant material was grown in field at Fargo location for seed increase in Summer 2005. This material was sufficient to test for dormancy in Fall 2005 greenhouse at two different temperatures and to conduct field trails in year 2006 at Prosper and Langdon locations.

Since the amount of seeds for Population II was less, the plant material was grown in field at Fargo location in year 2005 for seed increase. The seed thus obtained was tested for dormancy in Fall 2005 and Spring 2006 greenhouse seasons. These seeds were also grown in field at Prosper and Langdon locations for year 2006 to test for dormancy, other agronomic traits and for seed increase. The seed material obtained from 2006 field trails were grown in 2007 field trails at Prosper and Langdon locations to test for dormancy and other agronomic traits.

Experimental design and field evaluation

Since seed dormancy is greatly influenced by environmental factors such as temperature (Anderson et al., 1993), the plant material was grown in the field as well as in the greenhouse. In the field, the Population I along with checks was grown in Prosper and Langdon locations in 2006 with two replicates at each location. Lines and seventeen checks were sown as one row plots with 10 x 10 simple lattice design. While in greenhouse it was grown for two seasons, Fall 2005 and Spring 2006 with three replicates in each season as randomized complete block design (RCBD).

The Population II along with 9 checks was grown in field at Prosper and Langdon locations in 2006 and 2007 as 10 x 10 simple lattice design with two replications at each location and for each year. The seed material was also grown in the greenhouse in Fall 2005 and Spring 2006 with three replicates in each season as RCBD. The seeds obtained from both field and greenhouse for both populations were tested for dormancy by germinating seeds at specific temperature and moisture condition.

Evaluation of dormancy response

The spikes were harvested when they were physiologically mature (Hantt and Wych, 1982). Harvested spikes were left to dry for three days at room temperature to reduce the relative water content (RWC) to 8-12%. Moisture content in seeds is critical for seed dormancy, as high moisture content will break seed dormancy when stored at -20^{0}C (Paterson et al., 1989). To determine moisture content, drying experiments were performed and relative water content was calculated as described by Paterson et al. (1989). Relative water content (RWC) was determined by measuring fresh weight (FW) and dry weight (DW) of 10 seeds obtained from primary florets of two central spikelets of five different spikes. Fresh weight was measured immediately and dry weight after 48 hrs of incubating seeds at 94^{0}C. The following formula was used to calculate RWC.

$$RWC = (FW-DW) \times 100 / FW$$

To preserve dormancy, all uniformly dried seeds were hand threshed and stored at -20^{0}C, before the germination tests were conducted.

Test for dormancy was done by germinating 50 seeds per line per replicate at a specific temperature for 30 days. In order to identify the specific temperature at which the entire population was screened, the seeds from the parental lines were tested at varying temperatures from 10^{0}C to 27^{0}C. The

temperature of 27^{0}C showing significant variation between the dormant and the non dormant parent was used to screen the entire population.

The germination experiments were done as described by Nyachiro et al. (2002). The seeds were germinated by placing them on sterilized filter paper moistened with distilled water in 9 cm diameter petri dishes. The number of seeds germinating was recorded daily for 30 days however, data was presented at 10 day interval ie. day 1, 11, 21 and 30. Germination is defined as the pericarp rupture over the embryo.

Statistical analysis

Percentage of germination (PG) was used to determine the level of dormancy. The values were calculated using the following:

PG = {(total no. of germinated seeds/ 50) x 100}

The data was analyzed using SAP-Statistical Analysis Package (Hammond, 1992) and the Statistical Analysis System (SAS Institute, 1988). Data from each location was analyzed separately, using the average effective error variance as an error term. Variances from each location were tested for homogeneity using Levene's test for homogeneity of variance. If the variances were homogenous then the data from all the locations were combined. The combined analysis was done as randomized complete block design (RCBD), using the adjusted genotype means of each location. Each location for the field experiments and each season for the greenhouse experiments were used as replicates in combined analysis (Cochran and Cox, 1957).

Table 1. Pertinent expected mean squares for a single location

Sources of variation	Degree of freedom	Expected mean squares
Rep	r-1	_
Block (Rep)	r(k-1)	_
Genotype	k^2-1	$\sigma^2_e + k_1G^2$
Error	(k-1) (rk-k-1)	σ^2_e
Total	rk^2-1	_

r = number of replicates; k = size of lattice.

Table 2. Pertinent expected mean squares for combined environment analysis

Sources of variation	Degree of freedom	Expected mean squares
Location	e-1	_
Rep(Loc)	e(r-1)	_
Genotype	k^2-1	$\sigma^2_E + k_1 \sigma^2_{GL} + k_2G^2$
Genotype*Location	(e-1) (k^2-1)	$\sigma^2_E + k_1 \sigma^2_{GL}$
Error	(k-1) (rk-k-1)	σ^2_E
Total	e k^2-1	_

r = number of replicates; k = size of lattice; e = number of environments

If the genotypic mean squares were significant then the differences were calculated using Fisher's protected LSD.

LSD for single location will be, LSD= $t_{0.05/2, \text{df error}}\ \sqrt{}2MSE / r$

LSD for combined analysis will be, LSD= $t_{0.05/2, \text{df genotype*location}}\ \sqrt{}2MS_{GxL} / r \times e$

where MSE is mean square error; MS_{GxL} is mean square of genotype x environment; r is number of replicates; e is number of environments or locations; df is degree of freedom.

Molecular mapping

Total DNA was isolated from the young leaves of the three week old plants as suggested by Hossain et al. (2004). The molecular markers used in this study were those reported to map on Triticeae chromosomes 3A and 3B (Nelson *et al.*, 1995; Röder *et al.*, 1998). The BARC microsatellite markers mapped by Song et al. (2005) and eight restriction fragment length polymorphisms (RFLP) and five simple sequence repeats (SSR) mapped on chromosome 3A by Nalam et al. (2006) were also used to identify the QTL(s) for dormancy. Expressed sequenced tags (EST's) from rice markers linked to red color gene were also used to identify homologous wheat sequences, and primers were designed using "Primer 3" (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) for additional saturation mapping of critical regions. The markers showing polymorphism between the two parents were used to screen the entire population.

Genotypic data for the markers mapped on chromosome 3A and 3B was analyzed using MAPMAKER/EXP version 3.0b (Lincoln et al., 1992) to generate a linkage map, and map distance in centiMorgan units (cM) was calculated with the Kosambi function (Kosambi, 1944).

QTL analysis

The putative QTL for dormancy was identified by performing association studies between the phenotypic and marker data using MQTL software (Tinker and Mather, 1995). The MQTL performed simplified interval mapping (SIM) and simplified composite interval mapping (sCIM) for QTL analysis. Simple interval mapping evaluates the association between the trait values and the genotype values of a putative QTL between pairs of adjacent marker loci. The peak at significant plot was taken as the location of a putative QTL. Composite interval mapping not only evaluates the target QTL at multiple analysis points across each interlocus interval, it also analyses the effect of background QTLs or markers which are found to be associated with the trait and lie close to the other QTLs. The SIM and sCIM both were used in estimation of QTLs with main effects and QTL x E effects.

Walking distance of 1 cM and type 🗁 error rate of 5% was used for analyses (Tinker and Mather, 1995; Gonzalez., 2000). For SIM, threshold values were calculated using 1000 permutations. The real QTLs were identified as the ones showing significant peaks in both SIM and sCIM. The background makers were chosen at every 25cM along the chromosome to control background variance while performing sCIM. The QTL peaks outside the average distance between the background markers were considered as different QTLs. Significance of QTL regions was reported as test statistics (TS= n ln (RSSr/ RSSf), where n is number of observations, RSSr is residual sum of squares and RSSf is the residual sum of squares for the model without the effect being tested. The TS calculated over one environment is similar to the likelihood ratio (LOD score) when it is multiplied by 0.22 (Tinker and Mather, 1995).

RESULTS

Phenotypic analysis for seed dormancy on chromosome 3A

The seed germination experiments to estimate seed dormancy were performed for 30 days, since the material was highly dormant, and 100% germination was not attained. Data was recorded for all 30 days however, we reported it at 10 day intervals (ie. day 1, 11, 21 and 30), in order to study the trend for seed dormancy in the experimental population. The parental lines namely, LDN, LDN (Dic-3A) and LDN (Dic-3B) were germinated at temperatures ranging from 10^0C to 30^0C. The lower temperatures did not show any variation between the parents. At 27^0C the parents showed maximum variation for the dormancy trait. Therefore 27^0C was selected as the critical seed germination temperature to screen the populations and estimate seed dormancy.

The phenotypic frequency distribution at Prosper 2006 was done for day 1, 11, 21 and 30. At day 1 and 11 positive skewness was observed, however at day 21 and 30 the Population I was negatively skewed (Table 3). Positive skewness indicates that the population values were shifted towards the left side of the mean and vice versa for the negative skewness. Parents were significantly different from each other for the dormancy trait at all four days (Table 4). The population was more dormant at day 1, and a shift in the frequency distribution curve was observed as the number of days of germination progressed to day 30 (Figures 2 and 3). Even though the population moved from dormant to non dormant parent by day 30, the majority of the lines had similar phenotype to the dormant parent (Figure 3). Thereby indicating that population was more similar as the dormant parent.

For Langdon 2006 location, the population is positively skewed for day 1, 11 and 21 (Table 3). However, for day 30 the population is negatively skewed. Similar results were also observed for the Prosper location at day 30 (Table 3). The parents were significantly different from each other for the dormancy trait for all the four days (Table 4). Presence of a large number of transgressive segregants for dormancy at day 1, 11, 21 and 30 indicates that the population was more similar to the dormant parent rather than the non dormant one (Figures 4, 5, 6 and 7). However, there were some lines in between the dormant and the non dormant parent.

The genotypic mean squares for seed dormancy trait at Prosper and Langdon locations were significant for day 1, 11, 21 and 30 (Appendix A Table A1, A2, A3 and A4).

Table 3. Shapiro-Wilk's normality test for seed dormancy from LDN (Dic-3A) RICLs at field locations

	W:Normal		Pr<W		Skewness	
	Prosper	Langdon	Prosper	Langdon	Prosper	Langdon
Day 1	0.79	0.75	0.0001	0.0001	1.75	2.56
Day 11	0.97	0.94	0.1003	0.0005	0.04	0.24
Day 21	0.94	0.96	0.0006	0.0114	-0.7	0.02
Day 30	0.88	0.97	0.0001	0.0371	-1.25	-0.1

Table 4. Phenotypic means for percent germination of the parents, population and the checks at Prosper and Langdon locations

Genotypes	Prosper				Langdon			
	Day1	Day11	Day21	Day30	Day1	Day11	Day21	Day30
LDN(Dic-3A)	0	25	58	76	3	31	49	56
LDN 16	31	73	99	99	8	65	81	82
Population	6.2	40.3	70.2	83.6	2.8	39.2	53.0	58.6
Langdon	19	57	82	91	5	69	75	76
Divide	11	51	72	82	4	61	67	69
Grenora	16	67	95	97	2	46	48	48
Alkabo	43	90	96	98	6	59	69	72
Dilse	22	63	74	88	2	47	55	56
Pierce	18	66	89	95	2	53	64	65
Plaza	16	77	91	91	10	72	74	74
Lebsock	11	74	93	95	3	37	39	40
Mountrail	15	74	91	94	1	61	67	67
Maier	16	55	81	90	5	48	55	62
Belzer	10	72	88	92	1	52	55	56
Ben	12	64	81	81	2	44	44	44
Chahba88//B	9	37	65	75	3	19	22	22
RI4137	0	17	42	55	0	7	17	20
Lact12/Ka	10	70	84	91	2	26	27	27
LSD	11.1	21.8	25.0	25.4	5.6	24.4	26.6	29.4
CV	120	49	25	15	121	47	34	29

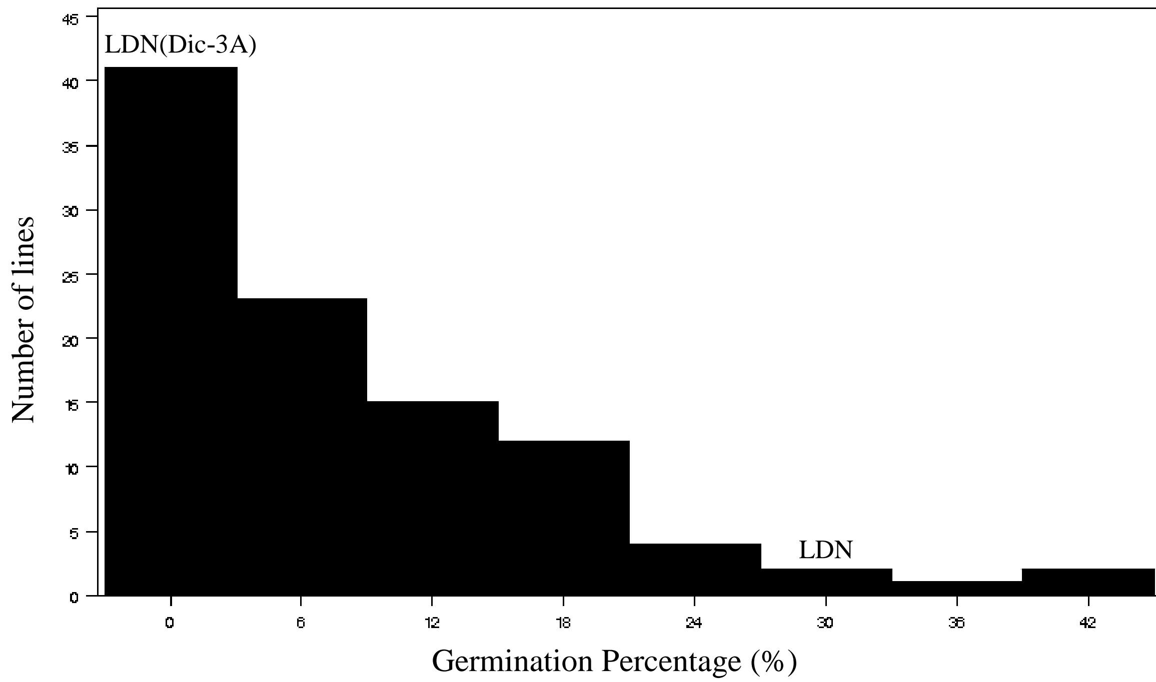


Figure 2. Frequency distribution for seed dormancy in LDN (Dic 3A) RICL population grown at Prosper in 2006 at 27^{0}C at Day 1.

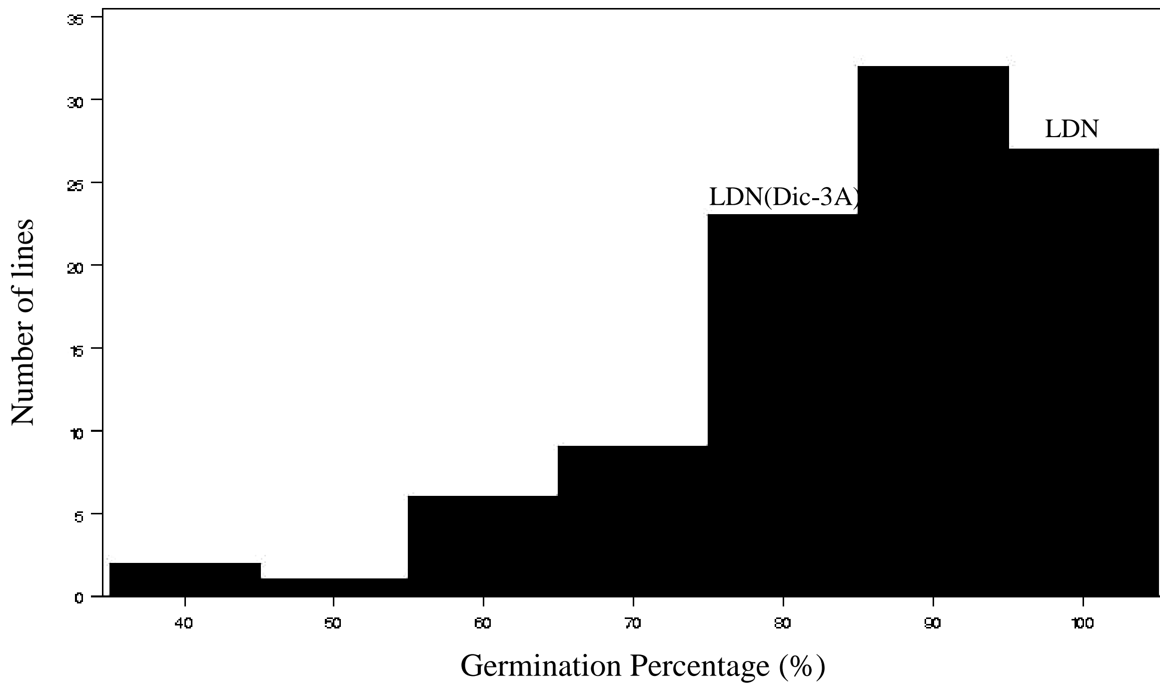


Figure 3. Frequency distribution for seed dormancy in LDN (Dic 3A) RICL population grown at Prosper in 2006 at 27^{0}C at Day 30.

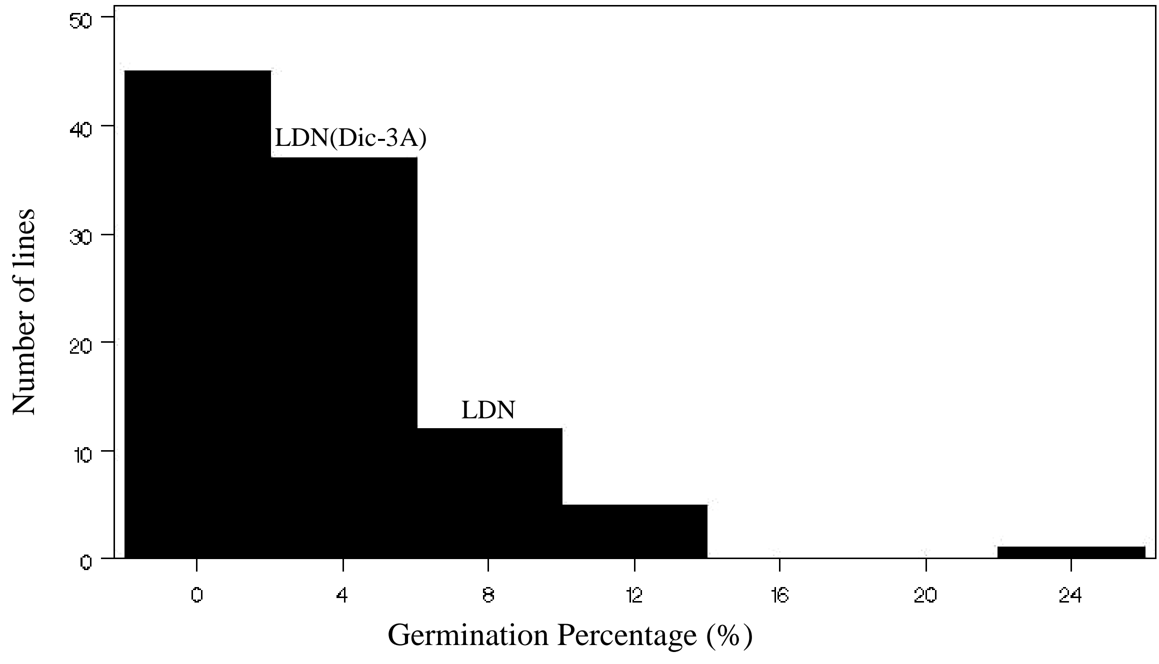


Figure 4. Frequency distribution for seed dormancy in LDN (Dic 3A) RICL population grown at Langdon in 2006 at 27^{0}C at Day 1.

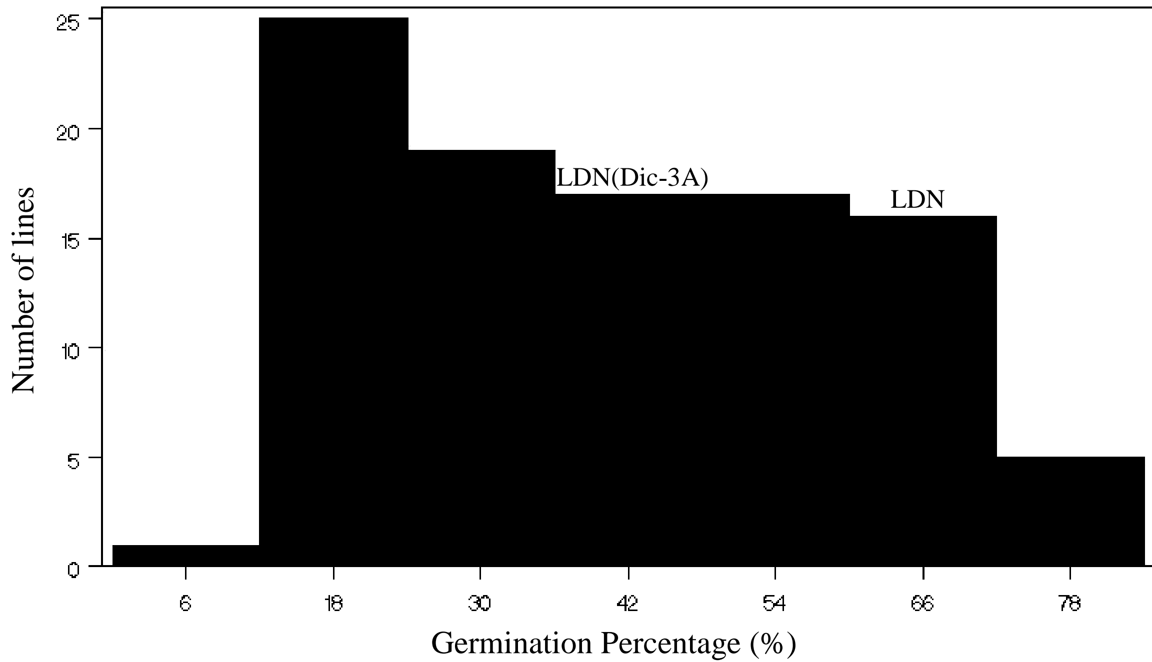


Figure 5. Frequency distribution for seed dormancy in LDN (Dic 3A) RICL population grown at Langdon in 2006 at 27^{0}C at Day 11.

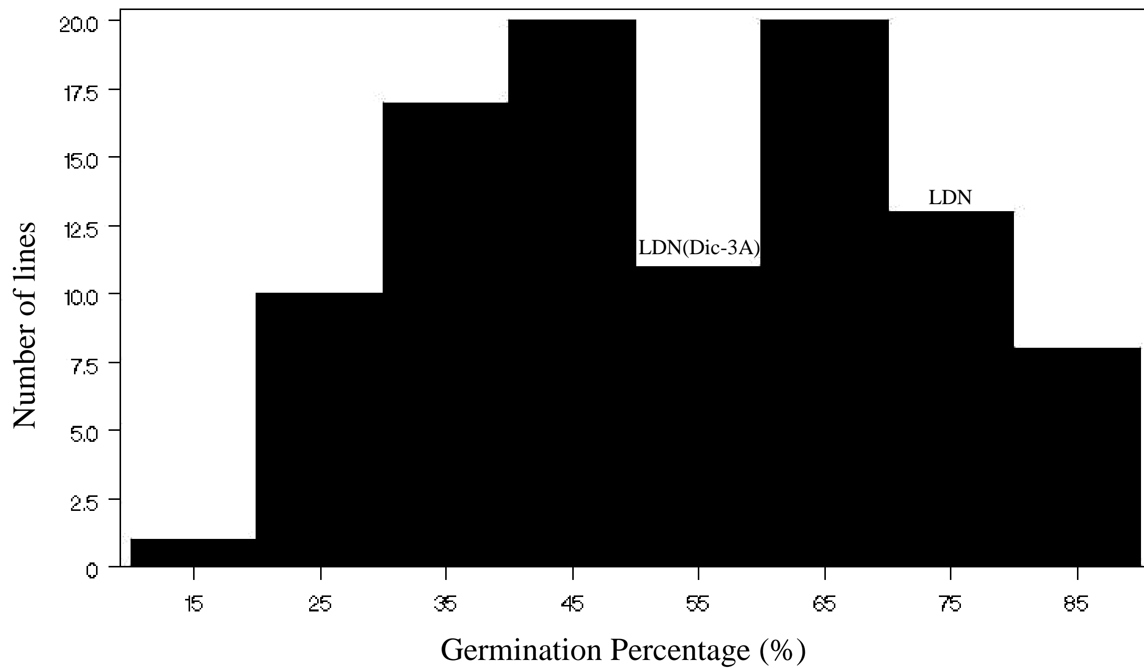


Figure 6. Frequency distribution for seed dormancy in LDN (Dic 3A) RICL population grown at Langdon in 2006 at 27^0C at Day 21.

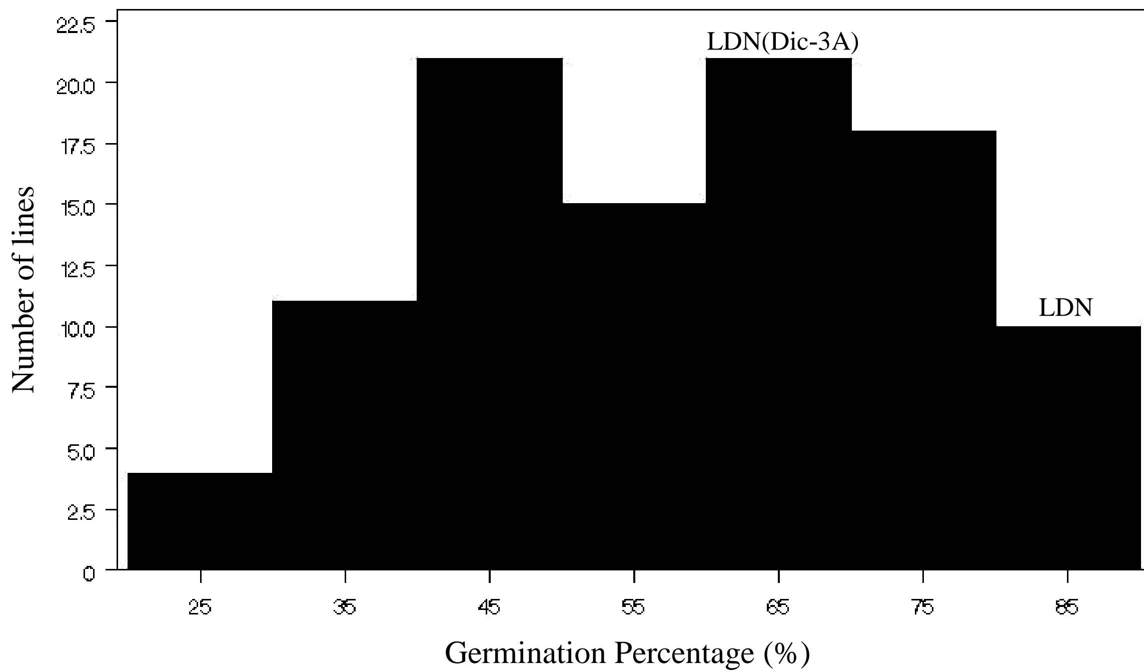


Figure 7. Frequency distribution for seed dormancy in LDN (Dic 3A) RICL population grown at Langdon in 2006 at 27^0C at Day 30.

However, the mean square values were found to be highest on day 11 for both the locations (Appendix A Table A2).

The Levene's test for homogeneity of variance over the Prosper and Langdon locations revealed that seed dormancy results are homogenous at day 11, 21 and 30, thus stating that the two locations could be combined for further analysis (Appendix B Table B1). However, seed dormancy observed on day 1 for both field locations was heterogeneous, thus could not be combined (Appendix B Table B1). The combined analysis did not find any genotype by environment interactions (GxE) for day 11, 21 and 30 (Appendix C Table C1).

For Spring 2005 material, the parents were not significantly different from each other at day 1 and 11 (Table 5). The population distribution moved from the dormant state to the non-dormant state by day 11, but behaved more as the non-dormant Langdon-16 parent (Figure 8). This is also evident from the negatively skewed population data present on day 11 (Table 6). Since 100% germination was obtained by day 11 for most of the lines in the population, the analysis of variance was not done for day 21 and day 30. The genotypic mean squares for dormancy were significant for day 1 but they were non significant for day 11 (Appendix A Table A5). This could be attributed to the 100% germination obtained by the day 11. For Fall 2005 material germinated at 20^0C the parents were significantly different in the dormancy pattern for day 11, 21 and 30 (Table 7). At day 1 the population was more dormant (Figure 9), but at day 11, 21 and 30 it became more similar to the non dormant parent (Table 7 and Figures 10, 11 and 12). This is also evident from the negative skewness of the data on day 30 (Table 5). Large numbers of transgressive segregants were observed for the non dormant parent on days 11, 21 and 30 (Figures 10, 11 and 12). Thus the population was more non dormant for this material.

When the Fall 2005 material was tested at 27^0C for dormancy, significant differences between the two parents was observed, however these differences were not greater than the ones obtained at 20^0C (Table 7). Population looked more similar to the non-dormant parent on day 11, 21 and 30 (Figures 13, 14 and 15). This is also evident from the negatively skewed data obtained for day 21 and 30 (Table 5). Transgressive segregants for both dormant and the nondormant parent were observed for day 21 and 30 (Figures 14 and 15).

Table 5. Shapiro-Wilk's normality test for seed dormancy for LDN (Dic-3A) RICLs for greenhouse (2005)

	W:Normal			Pr<W			Skewness		
	Fall 20^0C	Fall 27^0C	Spring	Fall 20^0C	Fall 27^0C	Spring	Fall 20^0C	Fall 27^0C	Spring
Day 1	0.53	0.2	0.92	0.0001	0.0001	0.0001	2.52	5.75	0.92
Day 11	0.96	0.96	0.56	0.02	0.035	0.0001	0.51	0.34	-3.44
Day 21	0.96	0.99	-	0.0319	0.78	-	0.08	-0.17	-
Day 30	0.95	0.95	-	0.0091	0.0064	-	-0.08	-0.75	-

Table 6. Phenotypic means for percent germination of parents and population for Spring greenhouse season

Genotype	Day1	Day11	Day21	Day30
LDN(Dic3A)	12.6	98	-	-
LDN	13.3	98.6	-	-
Population	27.3	97.2	-	-
LSD	13.7	-	-	-
CV	77.47	5.4	-	-

Table 7. Phenotypic means for percent germination of parents and population for Fall greenhouse season tested at 20^0C and 27^0C

Genotype	Fall 20^0C				Fall 27^0C			
	Day1	Day11	Day21	Day30	Day1	Day11	Day21	Day30
LDN(Dic3A)	0	5.3	6.6	6.6	0	5.3	25.3	37.3
LDN	0	24	34.6	41.3	0	16	41.3	61.3
Population	1.4	31.5	46.2	54.1	0.1	18.7	47.6	65.1
LSD	-	31.2	32.2	28.7	-	-	29.6	28.2
CV	215	58	47	42	506	61	39	28

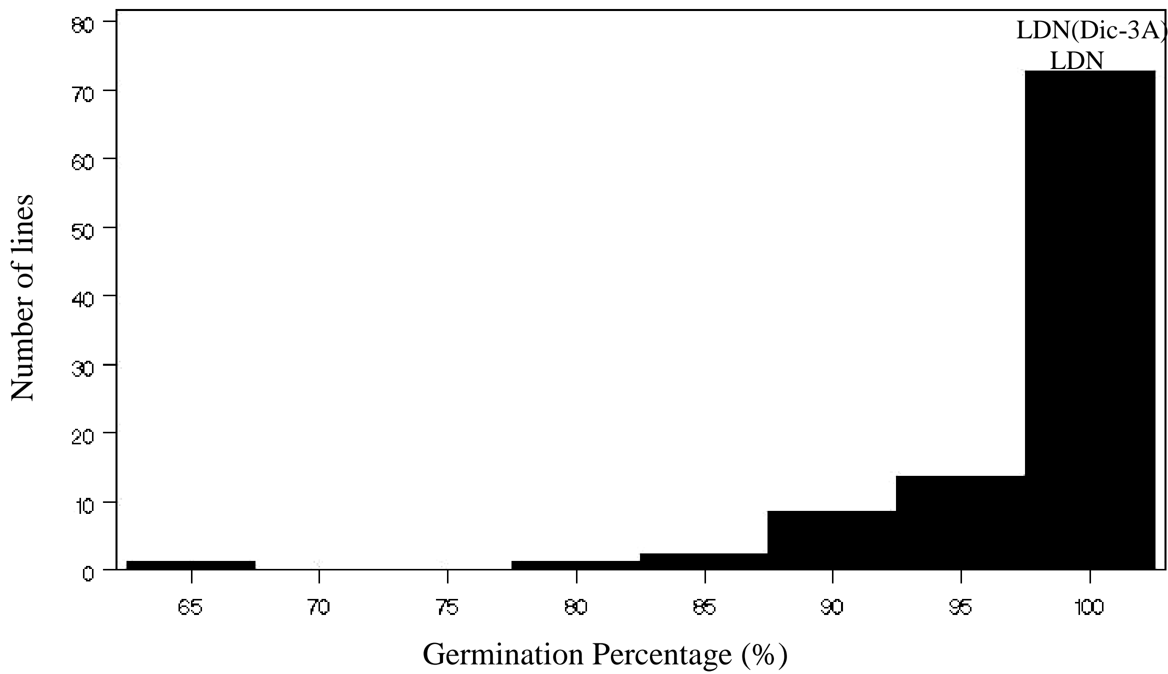


Figure 8. Frequency distribution for seed dormancy for LDN (Dic 3A) RICL population grown in the greenhouse in Spring 2005 at 20^0C at Day 11.

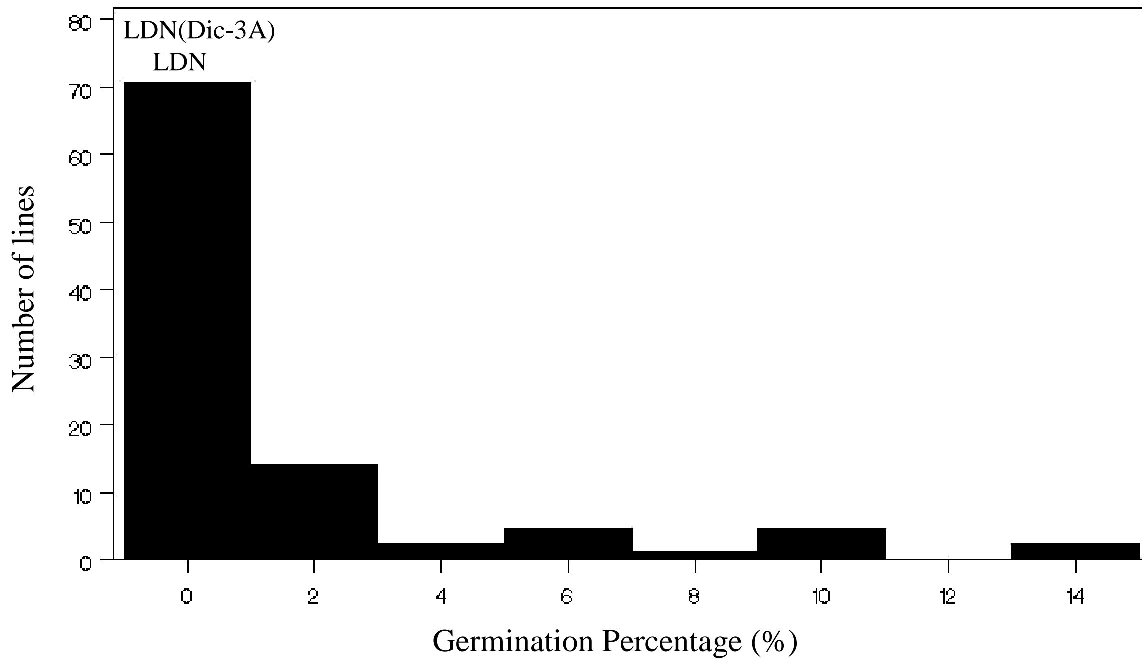


Figure 9. Frequency distribution for seed dormancy for LDN (Dic 3A) RICL population grown in the greenhouse in Fall 2005 at 20^0C at Day 1.

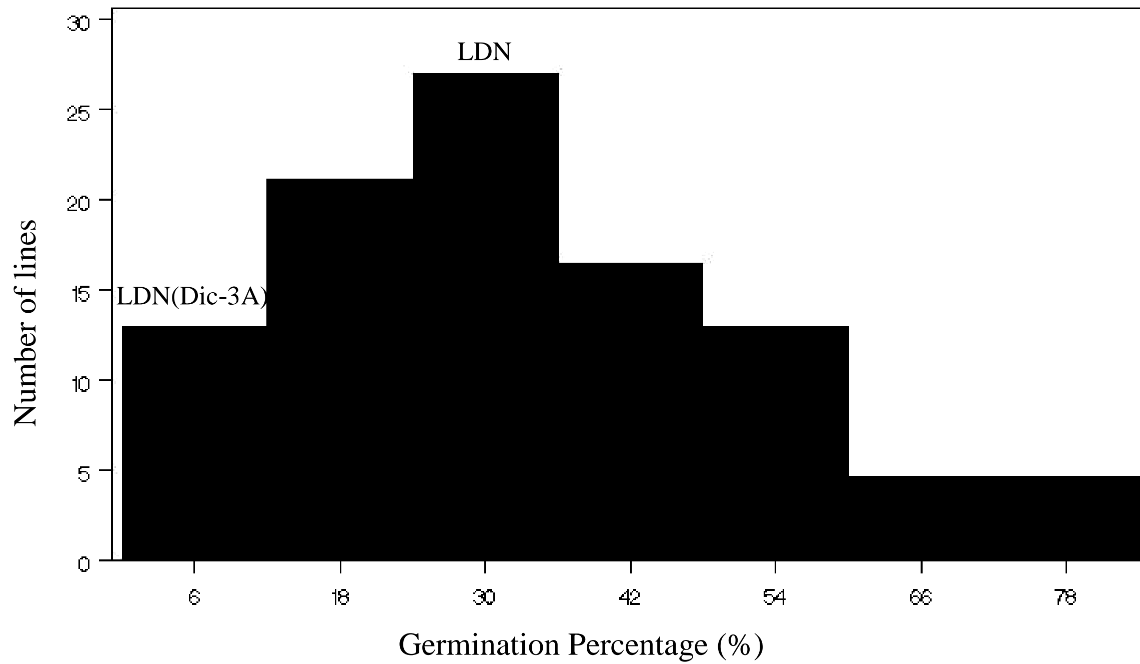


Figure 10. Frequency distribution for seed dormancy for LDN (Dic 3A) RICL population grown in the greenhouse in Fall 2005 at 20^{0}C at Day 11.

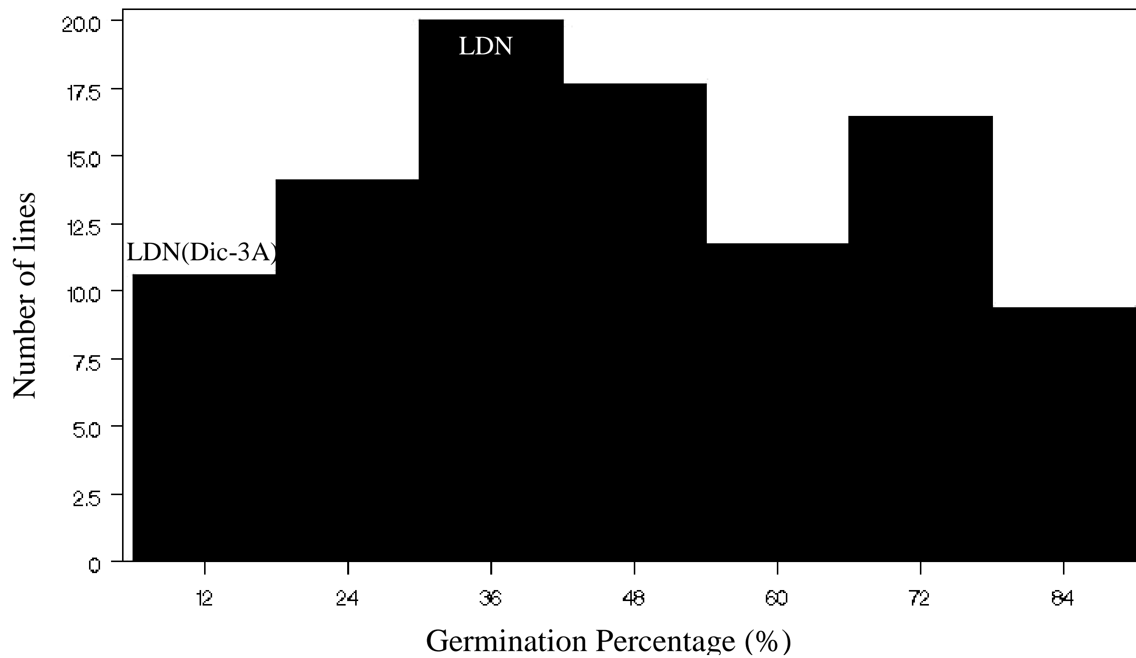


Figure 11. Frequency distribution for seed dormancy for LDN (Dic 3A) RICL population grown in the greenhouse in Fall 2005 at 20^{0}C at Day 21.

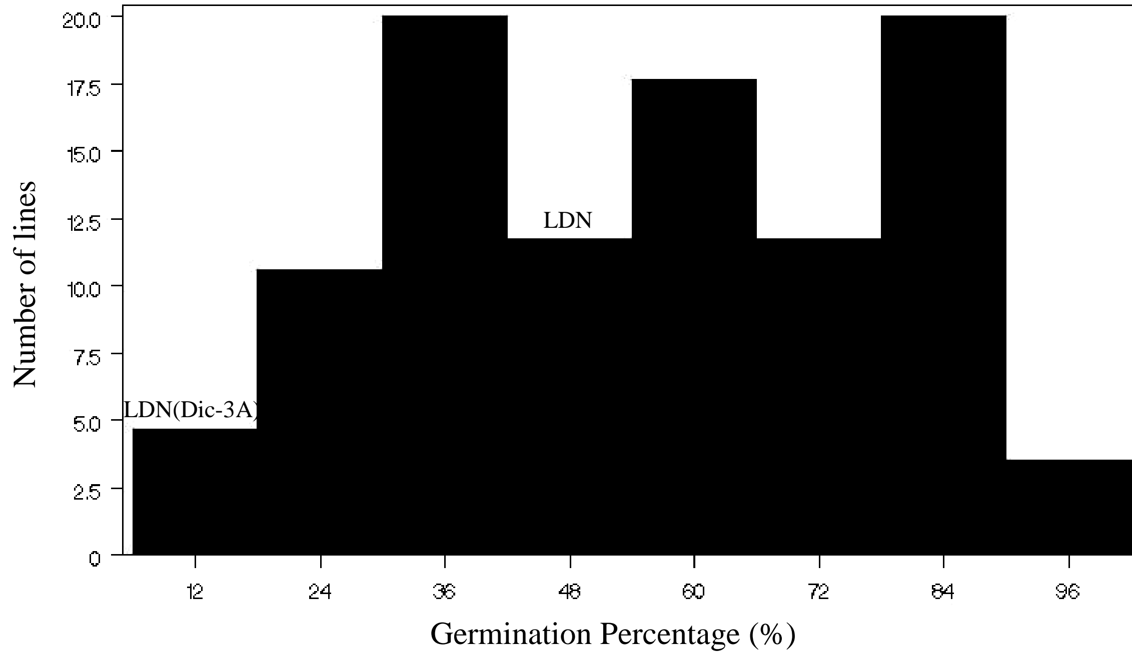


Figure 12. Frequency distribution for seed dormancy for LDN (Dic 3A) RICL population grown in the greenhouse in Fall 2005 at 20^0C at Day 30.

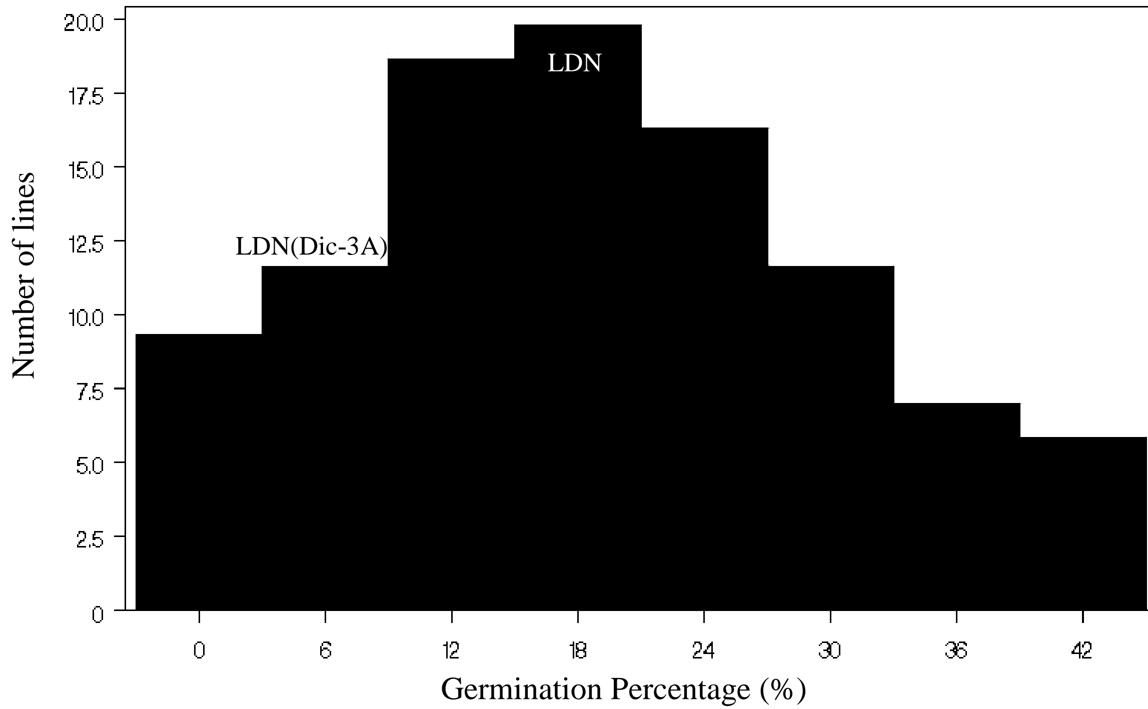


Figure 13. Frequency distribution for seed dormancy for LDN (Dic 3A) RICL population in Fall in 2005 at 27^0C at Day 11.

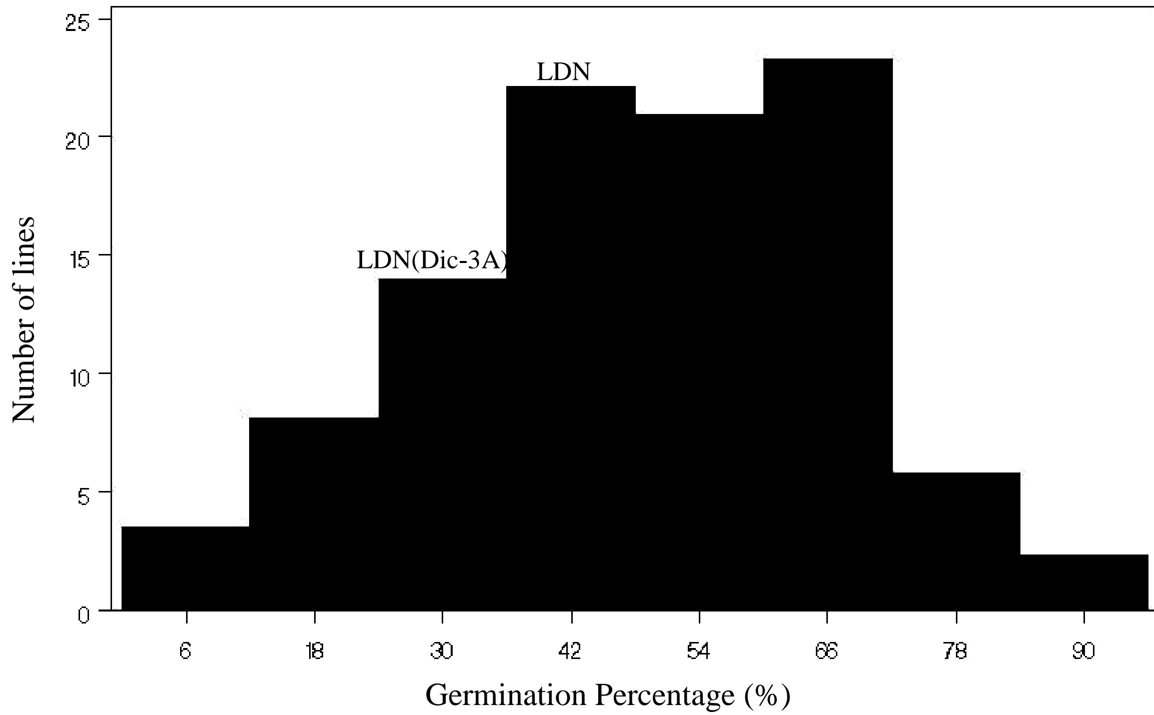


Figure 14. Frequency distribution for seed dormancy for LDN (Dic 3A) RICL population in Fall in 2005 at 27^0C at Day 21.

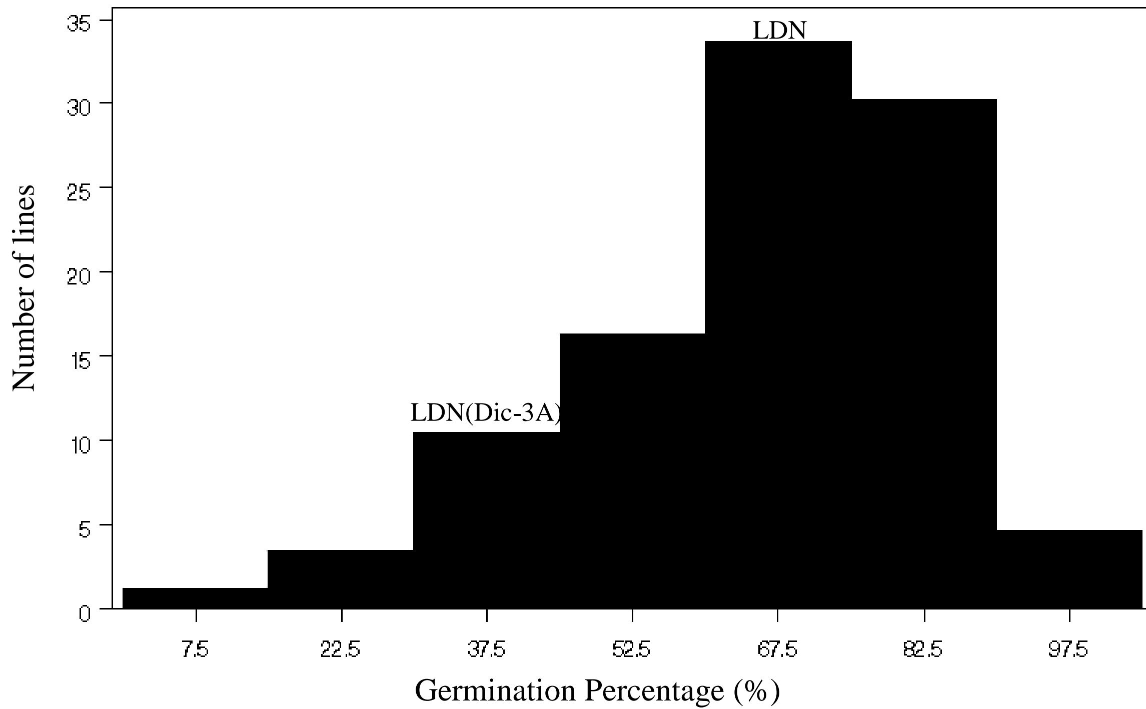


Figure 15. Frequency distribution for seed dormancy for LDN (Dic 3A) RICL population in Fall in 2005 at 27^0C at Day 30.

The genotypic mean squares were not significant at day 1 and significant for day 21 and day 30, when tested for seed dormancy at 20^{0}C and 27^{0}C for Fall material (Appendix A Table A6 and A7). However, the genotypic mean squares were significant at day 11 at 20^{0}C (Appendix A Table A6), while non significant at 27^{0}C (Appendix A Table A7). Variation in the population for seed dormancy was achieved by day 11 when tested at 20^{0}C (Appendix A Table A6), while at 27^{0}C it was achieved by day 21 (Appendix A Table A7). Thereby indicating, lower temperatures of 20^{0}C support reduced seed dormancy in the population. As the temperature increased to 27^{0}C the seed dormancy was increased. This can also be implicated from the genotypic mean square values that are much higher at 20^{0}C as compared to seed material tested at 27^{0}C (Appendix A Table A6 and A7).

The test for the homogeneity of variance between Spring and Fall seasons tested at both 20 and 27^{0}C revealed that the two seasons are heterogeneous (Appendix B Tables B2 and B3). Therefore the two seasons could not be combined for the further analysis and had to be treated separately.

Apart from dormancy, plant height and days to flowering traits were also obtained from Prosper and Langdon field locations for year 2006. The differences between the parents for both traits at both the locations were not significant (Table 8). For plant height, at both Prosper and Langdon locations, transgressive segregants were obtained for both dormant and non dormant parent (Figures 16 and 17). However, population was more skewed towards the nondormant parent. This is also evident from the negatively skewed results obtained for plant height for both Prosper and Langdon location (Table 9). For days to flowering the population was positively skewed (Table 9), thereby suggesting the population to be like the dormant parent (Figures 18 and 19).

The genotypes were significantly different for days to flowering trait at both Langdon and Prosper locations (Appendix A Table A8). Levene's test for homogeneity of variance revealed that the two field locations, Langdon and Prosper, are homogeneous and could be combined (Appendix B Table B4). The combined analysis did not show a significant GxE interaction for the trait (Appendix C Table C2).

The plant height trait showed significant genotypic differences for both Langdon and Prosper locations (Appendix A Table A9). For plant height the values were higher at Prosper location in comparison to the Langdon location (Appendix A Table A9). This could be attributed to the difference in the soil gradient and other environmental factors affecting plant height at the two locations.

Table 8. Phenotypic means for plant height and days to flowering of the parents, population and checks at Prosper and Langdon locations

Genotypes	Prosper		Langdon	
	HT	DOF	HT	DOF
LDN (Dic-3A)	60	109	57	119
LDN 16	64	120.5	61	126
Population	61.7	113.4	59.3	117.4
Langdon	65	127.5	63.5	132.5
Divide	60	92	60	103.5
Grenora	61	80.5	56.5	92
Alkabo	61	87	55	94
Dilse	62.5	90	60	92
Pierce	60	92.5	55.5	103.5
Plaza	61.5	85	57	130
Lebsock	60	87.5	54.5	104.5
Mountrail	61	92.5	57	107
Maier	61.5	86.5	57.5	102.5
Belzer	62	105	58.5	109
Ben	60	90	56	111
chahba88//B	60	73.5	55	78
RI4137	61	99	53	111
lact12/Ka	59	87.5	53.5	96.5
LSD	28.4	2	19.6	2.4
CV	10.7	3.9	8.7	4.6

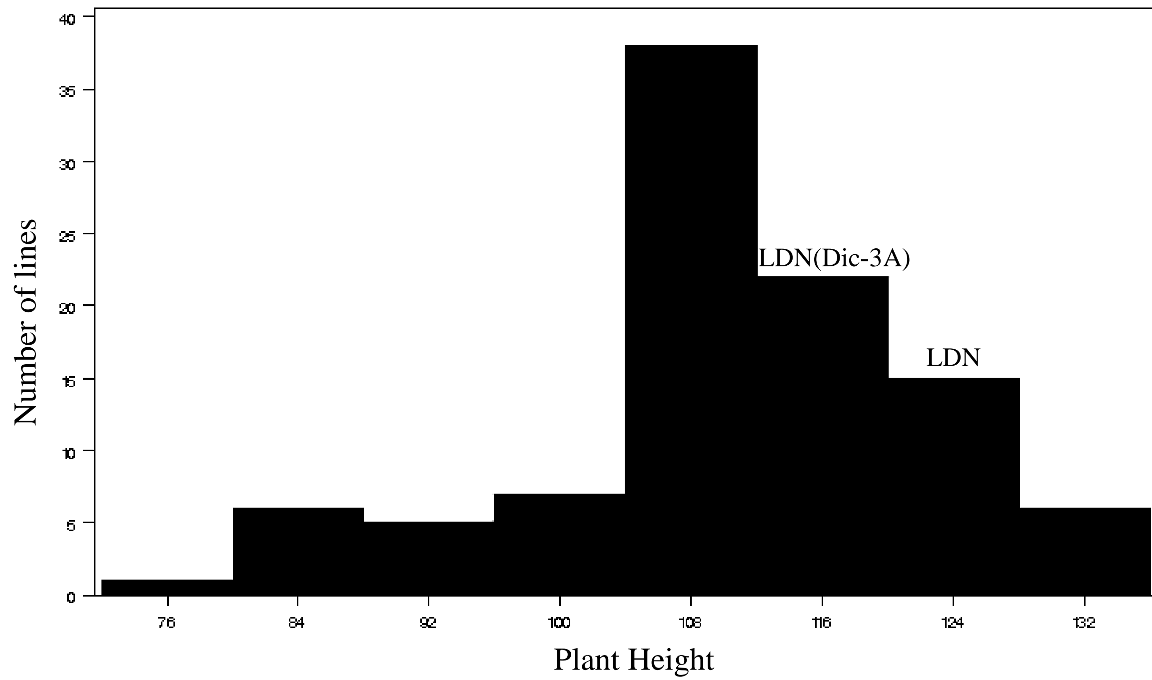


Figure 16. Frequency distribution for plant height for LDN (Dic 3A) RICL population at Prosper in 2006.

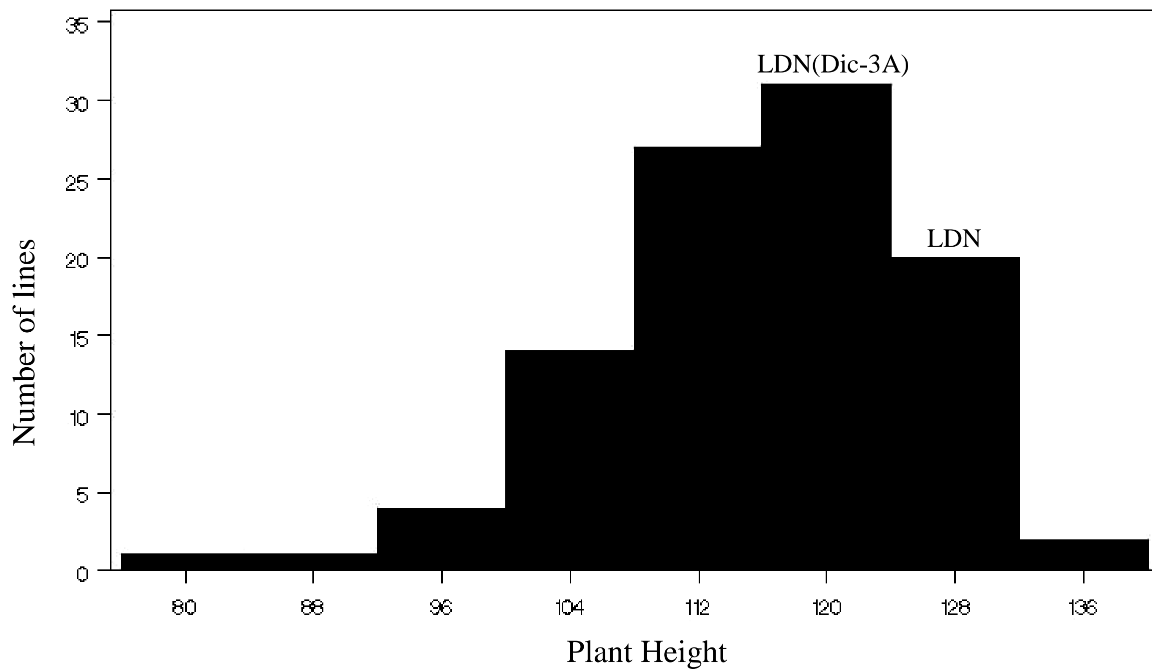


Figure 17. Frequency distribution for plant height for LDN (Dic 3A) RICL population at Langdon in 2006.

Table 9. Shapiro-Wilk's normality test for agronomic traits for LDN (Dic 3A) RICLs at field locations

	W:Normal		Pr<W		Skewness	
	Prosper	Langdon	Prosper	Langdon	Prosper	Langdon
Days to Flowering	0.74	0.9	0.0001	0.0001	1.84	0.87
Plant Height	0.96	0.96	0.0065	0.006	-0.53	-0.81

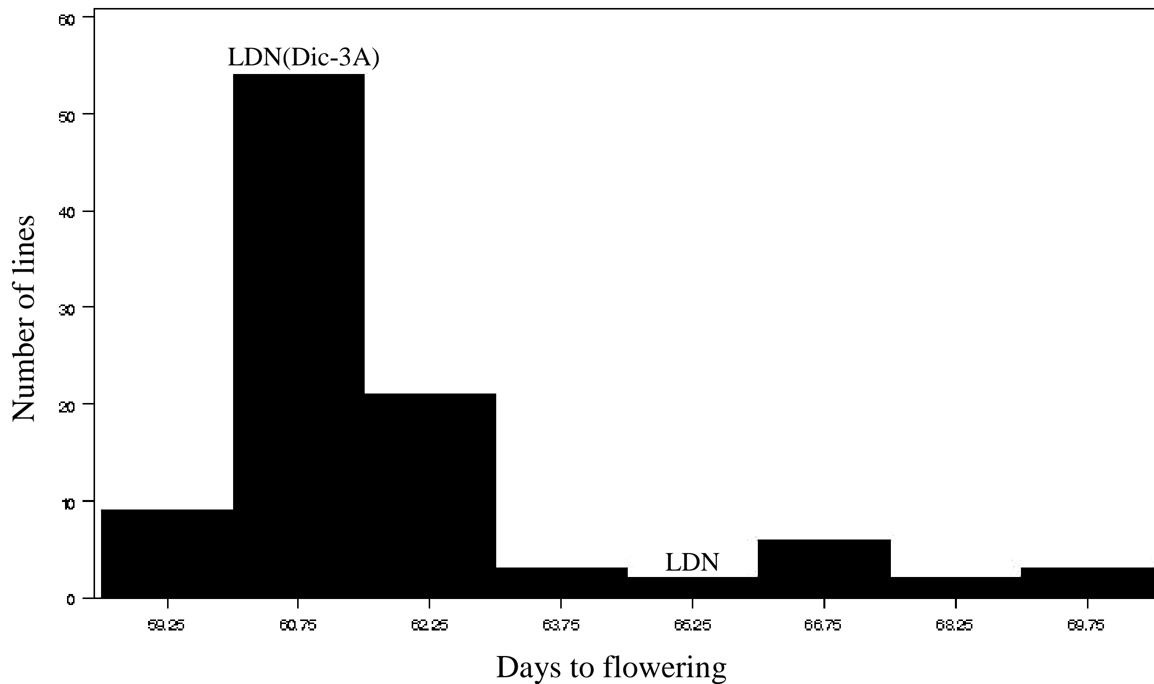


Figure 18. Frequency distribution for days to flowering for LDN (Dic 3A) RICL population at Prosper in 2006.

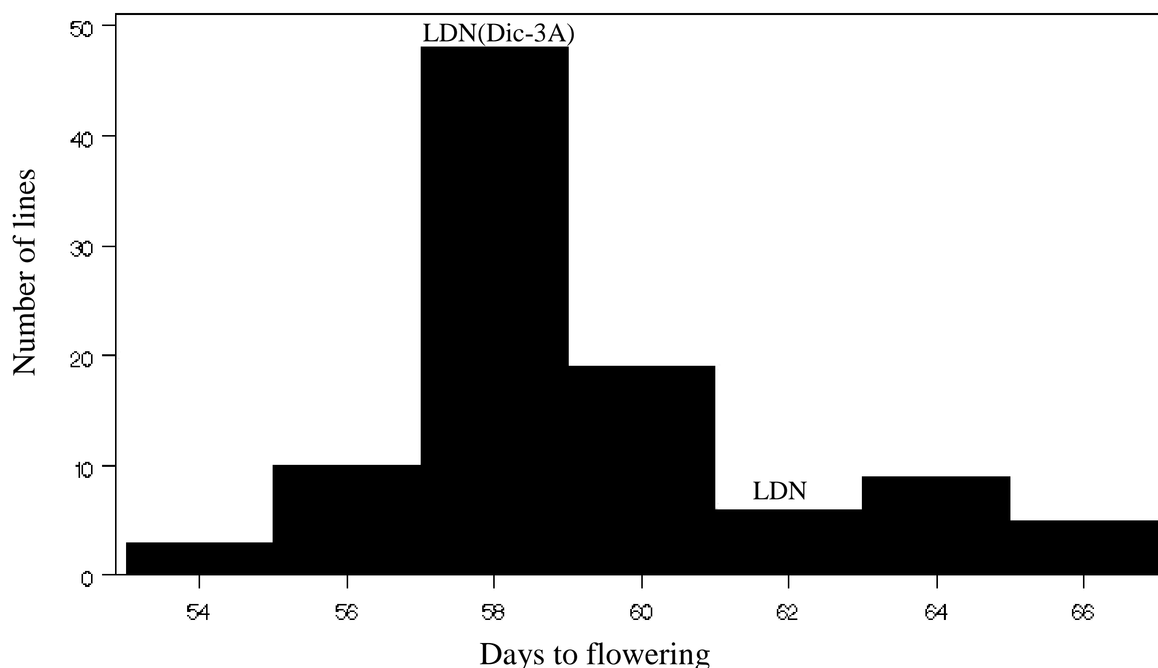


Figure 19. Frequency distribution for days to flowering for LDN (Dic 3A) RICL population at Langdon in 2006.

The test for homogeneity of variance suggested that the two locations are homogeneous and thereby could be combined (Appendix B Table B4). The combined analysis showed non significant GxE effect (Appendix C Table C2).

Phenotypic correlations for seed dormancy and other agronomic traits on chromosome 3A

Phenotypic correlations were calculated between the seed dormancy, plant height and days to heading traits as shown in Table 10. Seed dormancy did not show any correlation with the days to heading trait at either Prosper or Langdon locations. However, it was negatively correlated with plant height at day 1 and day 11 for the Prosper location and positively correlated for day 1, 11, 21, and 30 for the Langdon location. Plant height was also found to be positively correlated with days to heading trait at Prosper and Langdon locations. Correlation was also studied for the seed dormancy trait between 1, 11, 21 and 30 days to germination (Table 11). As a result, all days were positively correlated to each other. Day 1 was found to be significantly correlated with day 11 and day 11 showed highest correlations with day 21 for all the field locations. Similarly day 21 was significantly correlated with day 30 for all the field locations.

Table 10. Correlation coefficients for days to heading (DTH), plant height (HT) and seed dormancy recorded at day 1, 11, 21 and 30 for LDN (Dic 3A) RICL population

	Prosper		Langdon	
Days to germination	DTH	HT	DTH	HT
Day1	0.05	-0.28**	-0.13	0.18*
Day11	0.11	-0.20*	0.07	0.13
Day21	0.03	-0.11	0.12	0.25**
Day30	-0.07	-0.09	0.11	0.33**
DTH	1.00	0.55**	1.00	0.37**

* = significant at ≤ 5% level of significance
** = highly significant at ≤1% level of significance

Table 11. Correlation coefficients for seed dormancy recorded at 1, 11, 21 and 30 days to germination for LDN (Dic 3A) RICL population at field locations

	Prosper 2006				Langdon 2006			
	Day 1	Day 11	Day 21	Day 30	Day 1	Day 11	Day 21	Day 30
Day 1	1.00	0.78**	0.61**	0.49**	1.00	0.63**	0.51**	0.45**
Day 11	-	1.00	0.87**	0.7**	-	1.00	0.91**	0.81**
Day 21	-	-	1.00	0.91**	-	-	1.00	0.97**
Day 30	-	-	-	1.00	-	-	-	1.00

** = highly significant at ≤1% level of significance

Marker analysis for seed dormancy on chromosome 3A

Genetic linkage map published by Nalam et al. (2006) was used to identify quantitative trait loci (QTL) in the mapping population of 83 individuals derived from the cross between LDN x LDN (Dic-3A). Thirty one markers were used to generate the genetic linkage map of 252cM, with an average distance between each marker of 10cM (Figure 24, Page 69).

The association between the phenotypic data recorded from the field and the greenhouse season and the genotypic data was used to identify QTL for the dormancy trait. Single locus analysis revealed QTL for day 1, 11, 21 and 30 for all the locations separately and also for the combined locations. At day 1, three QTL were identified at Prosper location, spanning between the marker intervals Xcfa2037-*Xbcd115, Xcfa2193-Xcfd2a* and *Xwmc264-Xcfa2193,* explaining 17.73, 37.04 and 38.69% of phenotypic variation, respectively (Table 12). The QTL identified between the marker interval *Xcfa2193-Xcfd2a* was found to be associated with the red seed coat color trait and was also consistently present for day 11 and day 21, explaining 33.53 and 17.03% of variation for the dormancy trait collected at Prosper (Table 12). The red seed coat color has been associated with the dormancy trait in the previous studies reported by (Flintham and Gale, 1996; Imtiaz et al, 2008). However, this QTL was not significant for data at day 30 (Table 12). The QTL between the marker interval *Xcfa2037-Xbcd115* was also observed at day 11 explaining 22.91% of variation for the dormancy trait collected for Prosper (Table 12). However this QTL was not significant for the data at day 21 and 30.

The third QTL identified at day 11, between the marker interval *Xbarc294- Xbarc12*, was also observed on day 21 and day 30 at Prosper location and explained 16.83, 30.67 and 20.65 % of phenotypic variation for the dormancy trait, respectively (Table 12). QTL present between the marker interval *Xbcd828-Xcfa2134* and *Xcfa2134-Xcfa2037* were identified for day 21 and day 30, respectively, each explaining 13% of variation for the trait (Table 12). For the Prosper location three QTL were identified at day 1, 11 and 21, however, only two QTL were identified at day 30.

At Langdon location two QTL were identified at day 1 spanning between marker intervals *Xcfa2193-Xcfd2a* and *Xwmc264-Xcfa2193,* explaining 27.94 and 28.72% of variation for the trait, respectively (Table 12). The QTL identified between the marker interval *Xcfa2193-Xcfd2a* was consistently present for day 11, 21 and 30 and explained 55.28, 28.72 and 14.81 % of phenotypic

Table 12. The r^2 estimates for seed dormancy QTL detected at 1, 11, 21 and 30 days of germination for LDN (Dic-3A) RICLs for field trails using single locus analysis

Marker Interval		Prosper				Langdon			
		Day1	Day11	Day21	Day30	Day1	Day11	Day21	Day30
Xcfa2037-Xbcd115	QTL-1	17.7	22.9	-	-	-	-	-	-
*Xcfa2193-Xcfd2a**	QTL-2	37	33.5	17	-	27.9	55.2	28.7	14.8
Xwmc264-Xcfa2193	QTL-3	38.6	-	-	-	28.7	-	-	-
Xbarc294-Xbarc12	QTL-4	-	16.8	30.6	20.6	-	-	-	-
Xbcd828-Xcfa2134	QTL-5	-	-	13.7	-	-	-	-	-
Xcfa2134-Xcfa2037	QTL-6	-	-	-	13.4	-	-	-	-

variation for the dormancy trait, respectively. This QTL was associated with the red seed coat color and was also present at the Prosper location for day 1, 11 and 21 (Table 12).

For the Spring greenhouse season only one QTL was identified at day 1 between the marker interval *Xbcd2044-Xwmc264*, explaining 23% of phenotypic variation for the trait (Table 13). No QTL were identified at day 11, 21 and 30 for the Spring season. The Fall 2005 greenhouse material tested for dormancy at 20^0C showed two QTL between marker interval *Xbarc12-Xgwm369* and *Xgwm32-Xcfa2164,* to be present for day 11, 21 and 30 and explaining phenotypic variation for the dormancy trait ranging from 30 to 47% and 14 to 19%, respectively (Table 13).

Another QTL identified between marker interval *Xgwm2-Br-A2* was identified on day 21 and 30 and explained 21 and 19% the phenotypic variation for the trait, respectively (Table 13). The material grown in Fall 2005 greenhouse season and tested for dormancy at 27^0C, showed two putative QTL at day 30 between marker intervals *Xbarc294-Xbarc12* and *Xwmc153-Xbarc51*, each explaining 15% of phenotypic variation for the dormancy trait (Table 13). The former one was also observed for the Prosper location at day 11, 21 and 30 (Table 12). Since no QTL were observed for Plant height and Heading date, further analysis was not done for these traits.

Table 13. The r^2 estimates for seed dormancy QTLs detected at 1, 11, 21 and 30 days of germination for LDN (Dic-3A) RICLs for greenhouse seasons using single locus analysis

Marker Interval		Spring	Fall 20^0C			Fall 27^0C
		Day1	Day11	Day21	Day30	Day30
Xbcd2044-Xwmc264	QTL-7	23.65	-	-	-	-
Xbarc294-Xbarc12	QTL-4	-	-	-	-	14.81
Xbarc12-Xgwm369	QTL-8	-	30.5	36.96	47.38	-
Xgwm32-Xcfa2164	QTL-9	-	15.93	19.3	14.5	-
Xgwm2-Br-A2	QTL-10	-	-	21.98	19.69	-
Xwmc153-Xbarc51	QTL-11	-	-	-	-	15.93

Fall 20^0C indicates seed germination test done at 20C for fall greenhouse material
Fall 27^0C indicates seed germination test done at 27C for fall greenhouse material

To estimate the QTL by environment interaction (QTLxE), the analysis for the Prosper and the Langdon field locations for day 1, 11, 21 and 30 were combined. .A major putative QTL found to be linked with the red seed coat color and spanning between the marker interval *Xcfa2193-Xcfd2a* identified for day 1, 11, 21 and 30 (Figure 20, 21, 22, and 23), explained 11.6 to 43.7% of phenotypic variation for the dormancy trait (Table 14).

The QTLxE effect was observed for this QTL at day 1, thereby suggesting that the QTL might not respond evenly across different locations at day 1 (Table 14). The second QTL spanning between the marker interval *Xbarc12-Xgwm369* was identified on day 11, 21 and 30 (Figure 21, 22 and 23), explaining 11.4 to 18.9% of phenotypic variation for the dormancy trait (Table 14). QTLxE interaction was observed on day 21 for this QTL (Table 14). Two more QTL spanning between the marker intervals *Xbarc45 -Xgwm2* and *Xcfa2134-Xcfa2037* were identified for day 21 and 30 (Figure 22 and 23), each explaining 16 and 15% of variation for the dormancy trait, respectively (Table 14).

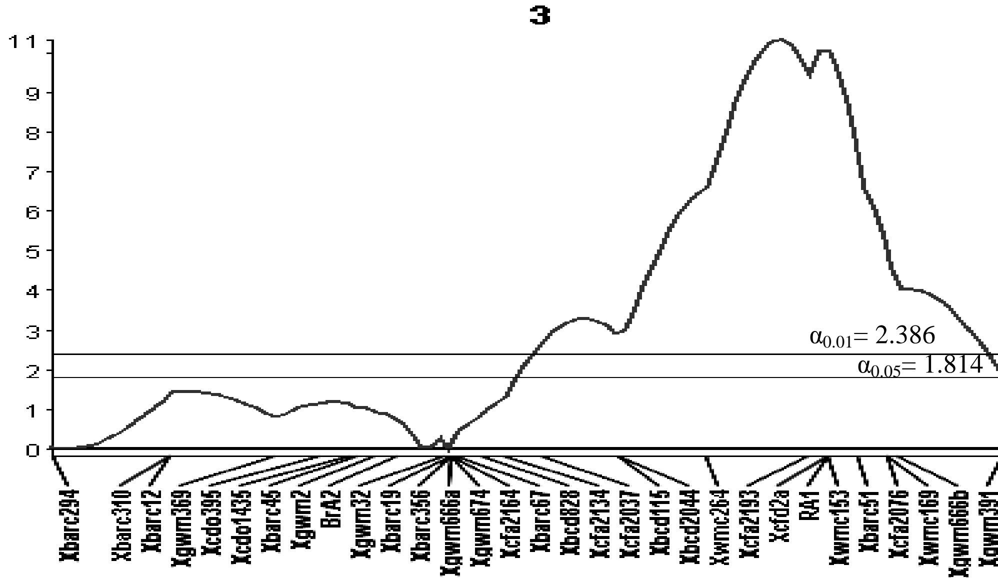


Figure 20. QTL peaks for seed dormancy at Day 1 for LDN (Dic-3A) RICLs for combined field locations using SIM. Peaks above the LOD score at 0.01 and 0.05 level of significance calculated by 1000 permutations, are putative QTL.

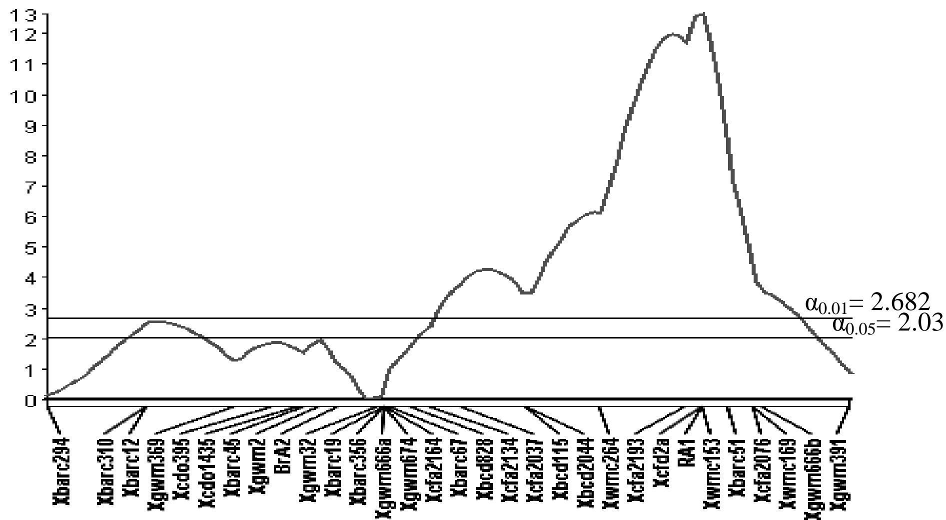


Figure 21. QTL peaks for seed dormancy at Day 11 for LDN (Dic-3A) RICLs for combined field locations using SIM. Peaks above the LOD score at 0.01 and 0.05 level of significance calculated by 1000 permutations, are putative QTL.

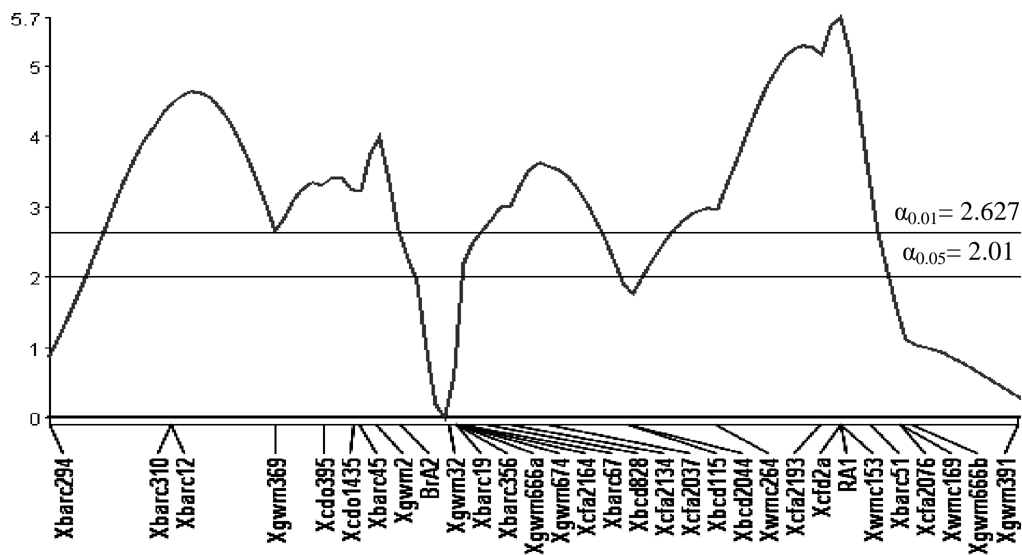


Figure 22. QTL peaks for seed dormancy at Day 21 for LDN (Dic-3A) RICLs for combined field locations using SIM. Peaks above the LOD score at 0.01 and 0.05 level of significance calculated by 1000 permutations, are putative QTL.

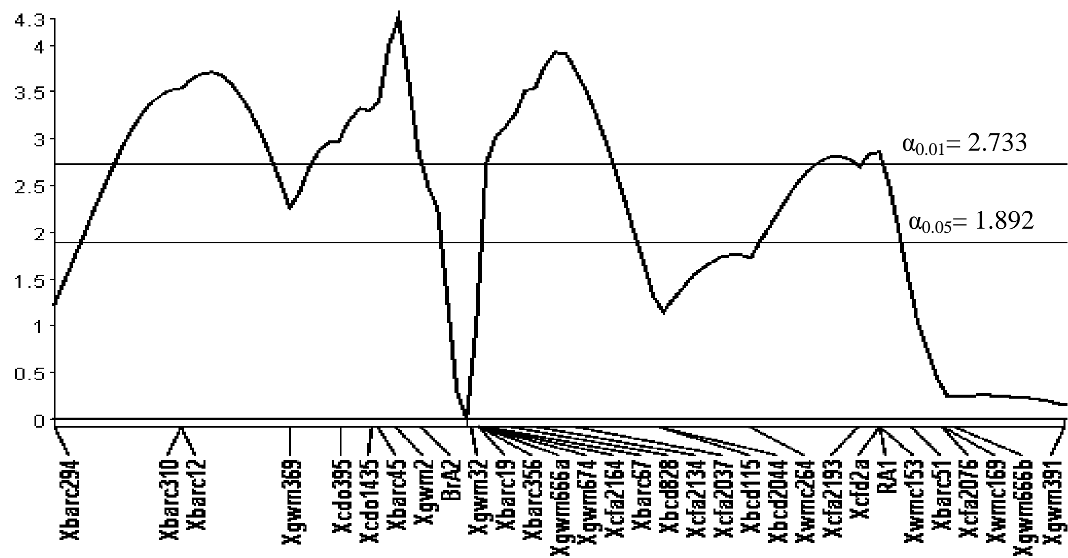


Figure 23. QTL peaks for seed dormancy at Day 30 for LDN (Dic-3A) RICLs for combined field locations using SIM. Peaks above the LOD score at 0.01 and 0.05 level of significance calculated by 1000 permutations, are putative QTL.

Table 14. The r^2 (%) estimates for QTL detected at day 1, 11, 21 and 30 for combined field location analysis (Langdon and Prosper) for LDN (Dic-3A) RILs

Marker Interval		Day1*	Day11	Day21*	Day30
Xcfa2037 - Xbcd115	QTL-1	11.8 (4.5)	18.4	-	-
Xcfa2193 - Xcfd2a	QTL-2	30.2 (8.4)	43.7	22.6	11.6
Xbarc12 - Xgwm369	QTL-8	-	11.4	18.9 (2.3)	14.3
Xbarc45 - Xgwm2	QTL-12	-	-	16.6	16.6
Xcfa2134 - Xcfa2037	QTL-6	-	-	15.2	15.2
Multilocus estimates		47	74	64	50

* Values in parenthesis are values for QTLx E effect

None of these QTL showed QTLx E interaction, thereby suggesting that these QTL were consistently present in different environments. A QTL between the marker interval *Xcfa2037-Xbcd115* was identified on day 1 and 11, contributing 11 to 18% of its phenotypic variation towards the total variation for the dormancy trait and also showed QTLxE interaction (Table 14).

Multilocus analysis was performed, for the QTL identified by combined location analysis, to estimate the total effect of all the QTL identified on day 1, 11, 21 and 30. Two QTL were identified on day 1, spanning between the marker interval *Xcfa2037-Xbcd115* and *Xcfa2193-Xcfd2a*, together contributing 47% of phenotypic variation for the dormancy trait (Table 14). Three QTL identified on day 11 accounted for 74% of total phenotypic variation for the trait and four QTL identified on day 21 and 30 accounted for 64 and 50% of phenotypic variation for the dormancy trait (Table 14).

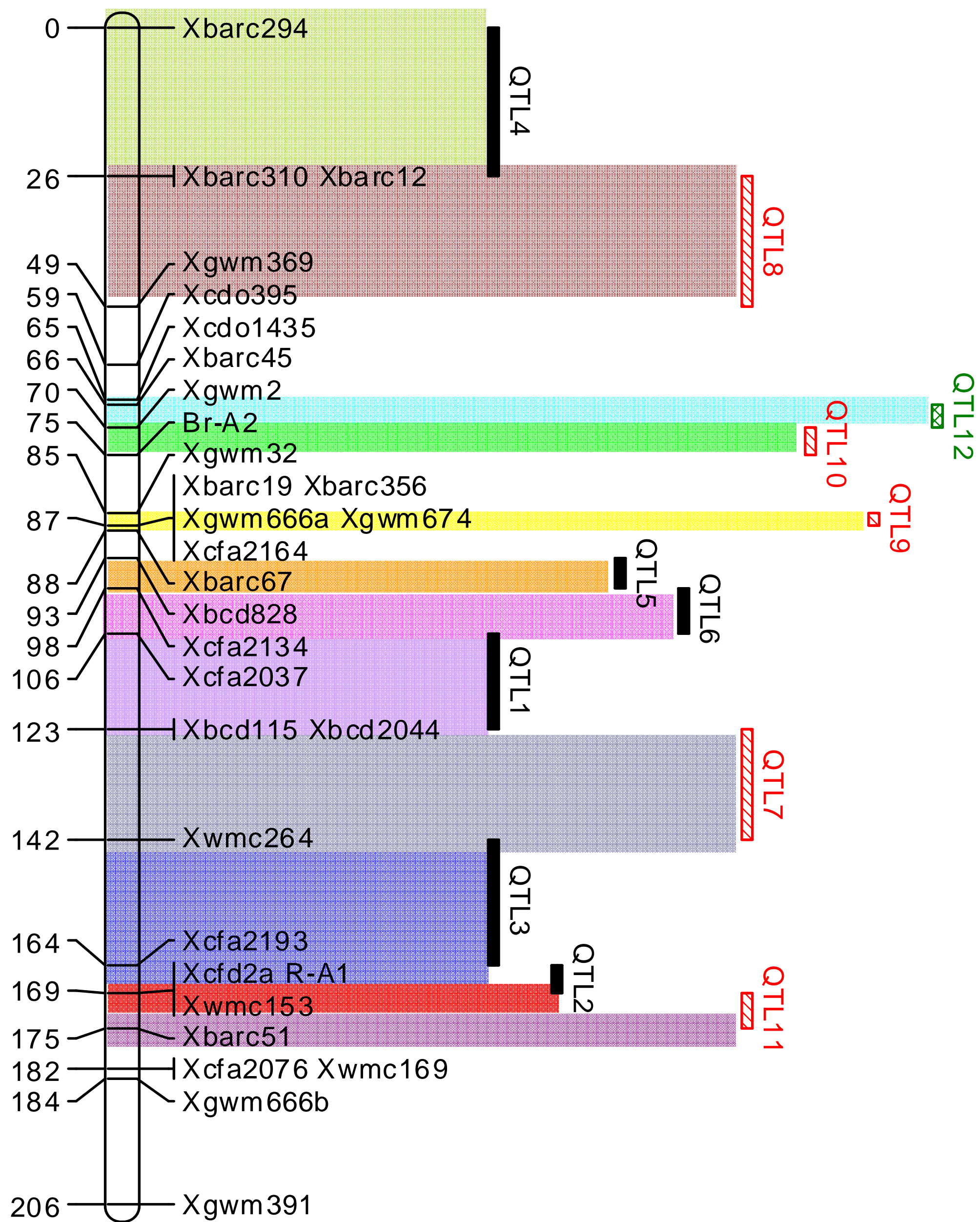


Figure 24. The linkage map of chromosome 3A constructed for the LDN (Dic-3A) RICLs. Solid black lines, red diagonal lines and green checked lines indicate QTL as referred in tables 12, 13 and 14 respectively.

Phenotypic analysis for seed dormancy on chromosome 3B

The population derived from the cross between LDN x LDN (Dic-3B) was analyzed for seed dormancy trait at Prosper and Langdon locations for 2006 and 2007 years and in greenhouse for Fall 2005 and Spring 2006. Other agronomic traits such as plant height and days to flowering were also recorded for Prosper and Langdon locations for both 2006 and 2007 years.

The frequency distribution pattern obtained for the Prosper 2006 location did not show significant differences between the two parents for day 1, 11, 21 and 30 for the dormancy trait (Table 15 and Figure 25, 26, 27 and 28). The transgressive segregants were observed for both dormant and non-dormant parents but the population was negatively skewed for day 11, 21 and 30 (Table 16). Thereby, indicating that the population was more like the non-dormant parent. For the Langdon 2006 location the parents did not show significant difference between each other for the dormancy trait (Table 15). The population seemed to be more dormant on day 1 and moved towards non-dormant parent by day 30 (Figure 29 and 32). This could be explained by the shift in the frequency distribution curve from day 1 to day 30. Thereby, suggesting that the population was more skewed towards the non-dormant parent for day 11, 21 and 30 (Table 16 and Figure 30, 31 and 32). This is evident from the negatively skewed data obtained for all three days (Table 16). At day 1 transgressive segregants were observed for both the parents (Figure 29). However, as the days for germination progressed to day 30 more transgressive segregants were observed for the dormant parent (Figure 32). The genotypic means squares were highly significant for the population grown at both Prosper and Langdon locations for year 2006 for the four days, however day 11 showed maximum genotypic mean square values for the seed dormancy trait at both locations (Appendix D. Table D1, D2, D3 and D4).

For Prosper 2007 location the parents did not show significant differences between each other for the dormancy trait at any given day (Table 17). However, the population showed similar distribution pattern as the Prosper 2006 and Langdon 2006 locations (Figure 33, 34, 35 and 36). The population was more skewed towards the non-dormant parent for day 11 and 21, although large number of transgressive segregant were also observed for the dormant phenotype for day 11, day 21 and day 30 (Table 18 and Figure 34, 35 and 36). The plant material obtained from Langdon 2007 location did not show significant difference between the parents for the dormancy trait (Table 17).

Table 15. Phenotypic means for percent germination of the parents, population and checks at Prosper and Langdon locations for year 2006

Genotype	Prosper 2006				Langdon 2006			
	Day1	Day11	Day21	Day30	Day1	Day11	Day21	Day30
LDN Dic 3B	12	66	93	100	27	77	84	84
Langdon	25	59	83	89	27	95	99	99
Population	13.5	48.1	71.8	81.8	19.1	70.1	76.9	78.5
Divide	10	56	78	88	7	75	79	82
Lebsock	45	89	97	100	7	80	80	80
Mountrail	16	71	93	97	11	67	67	67
Ben	14	66	80	82	16	60	62	62
Chahba88//B	8	39	43	45	12	49	53	54
RI4137	0	28	65	69	1	17	22	23
Iact12/Ka	26	83	92	96	20	74	74	75
LSD	15.6	21.9	20.6	18.5	20.1	24	20	21
CV	76.7	46.2	27.1	20	74.4	34.7	27.1	25.5

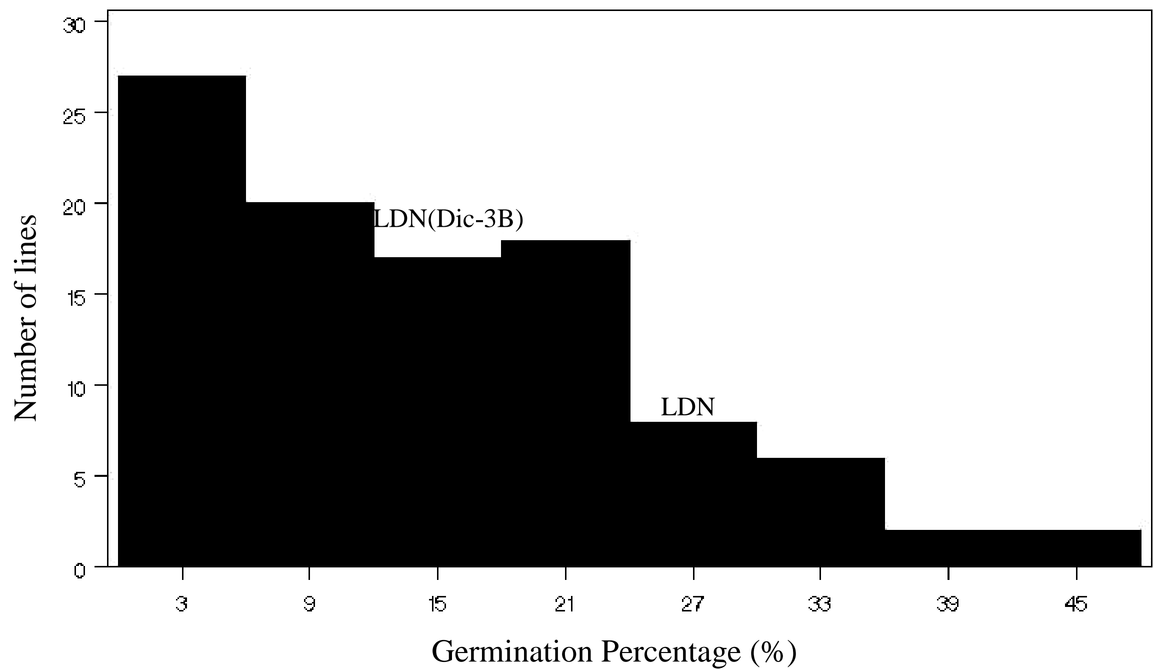


Figure 25. Frequency distribution of seed dormancy for LDN (Dic-3B) RICL population for Prosper 2006 material at day 1 germination test.

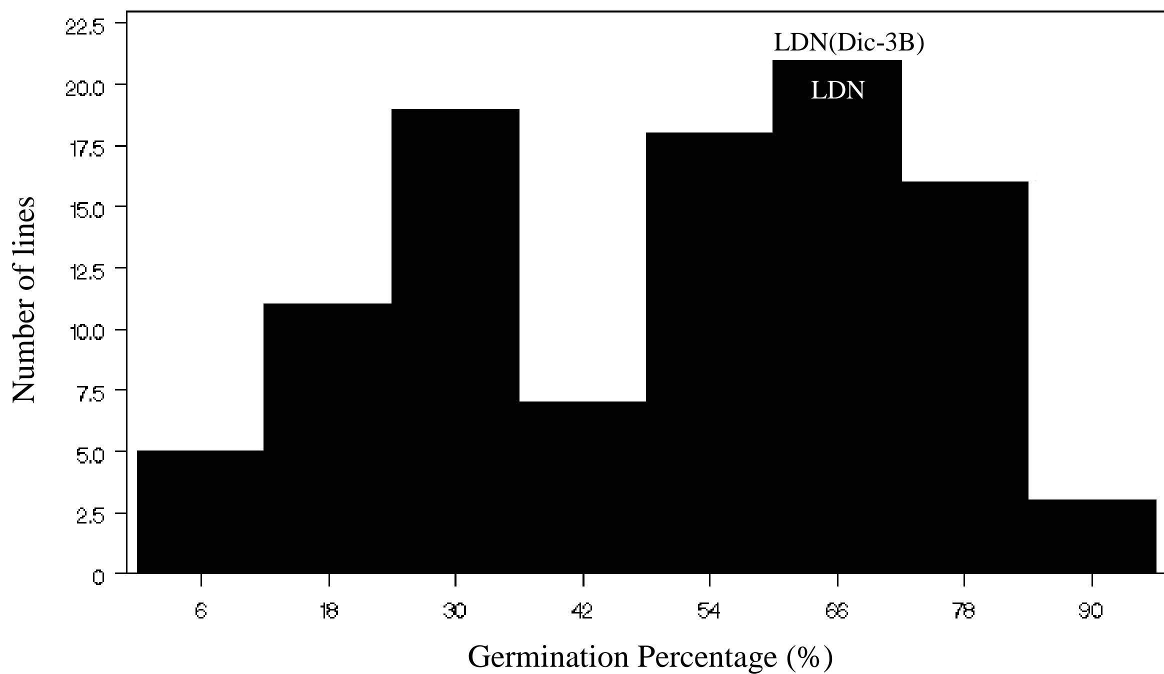


Figure 26. Frequency distribution of seed dormancy for LDN (Dic-3B) RICL population for Prosper 2006 material at day 11 germination test.

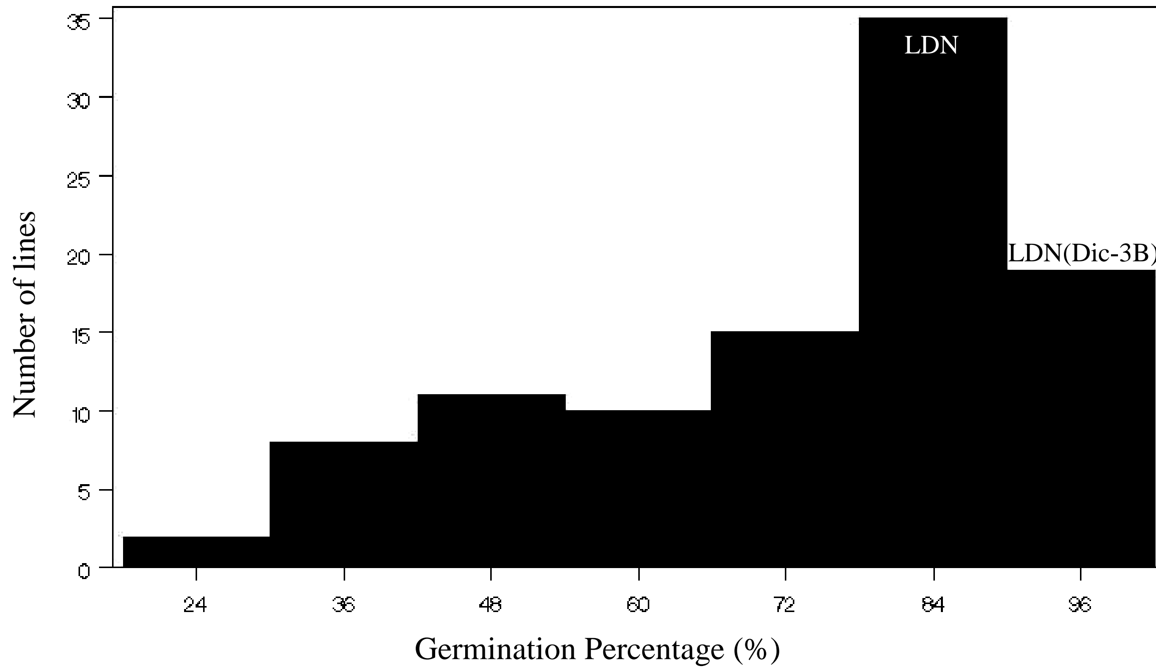


Figure 27. Frequency distribution of seed dormancy for LDN (Dic-3B) RICL population for Prosper 2006 material at day 21 germination test.

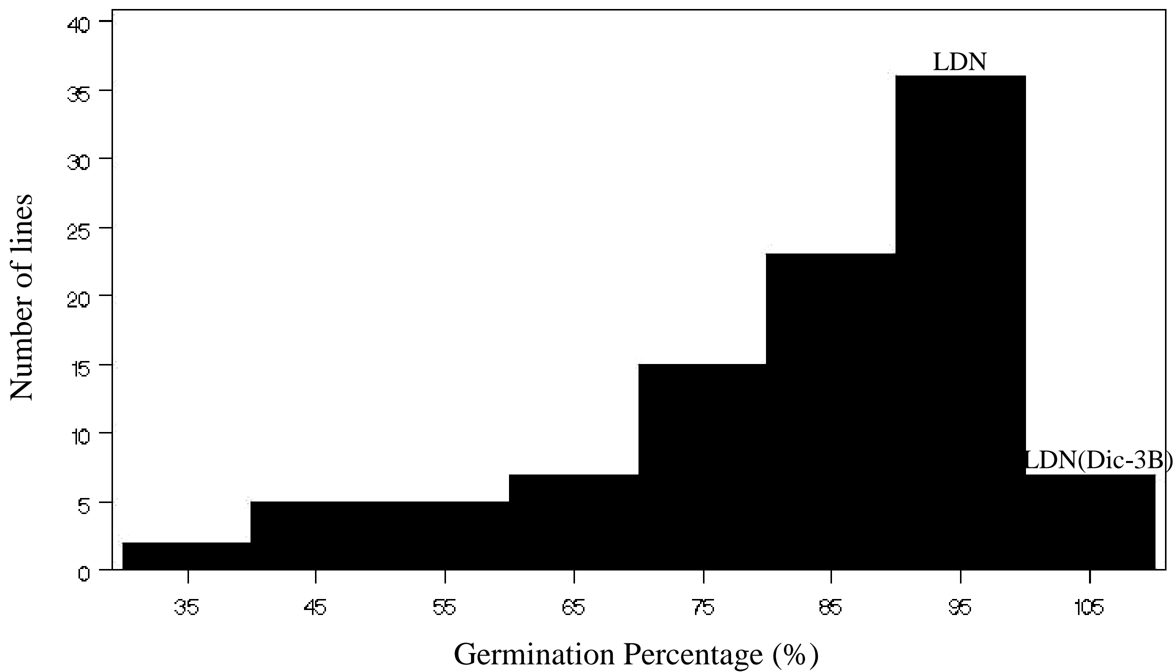


Figure 28. Frequency distribution of seed dormancy for LDN (Dic-3B) RICL population for Prosper 2006 material at day 30 germination test.

Table 16. Shapiro-Wilk's normality test for seed dormancy for LDN (Dic-3B) RICLs for 2006 field locations

	W:Normal		Pr<W		Skewness	
	Prosper	Langdon	Prosper	Langdon	Prosper	Langdon
Day 1	0.94	0.93	0.0002	0.0001	0.69	0.29
Day 11	0.94	0.87	0.0003	0.0001	-0.21	-0.72
Day 21	0.89	0.87	0.0001	0.0001	-0.86	-0.87
Day 30	0.87	0.86	0.0001	0.0001	-1.19	-0.94

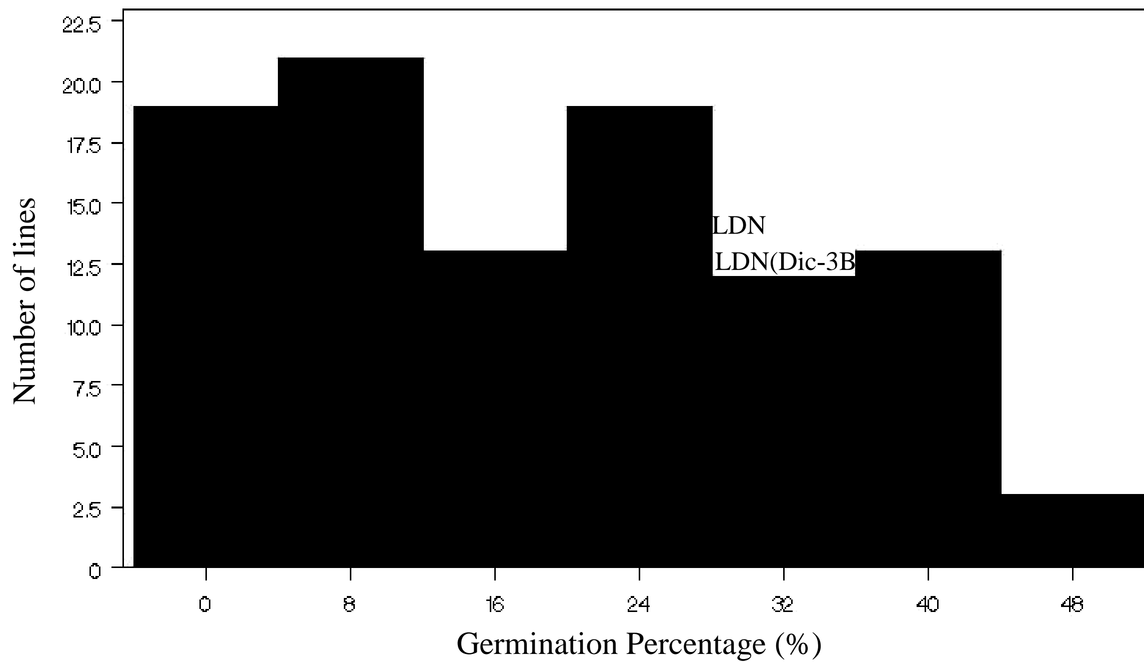


Figure 29. Frequency distribution of seed dormancy for LDN (Dic-3B) RICL population for Langdon 2006 at day 1 germination test.

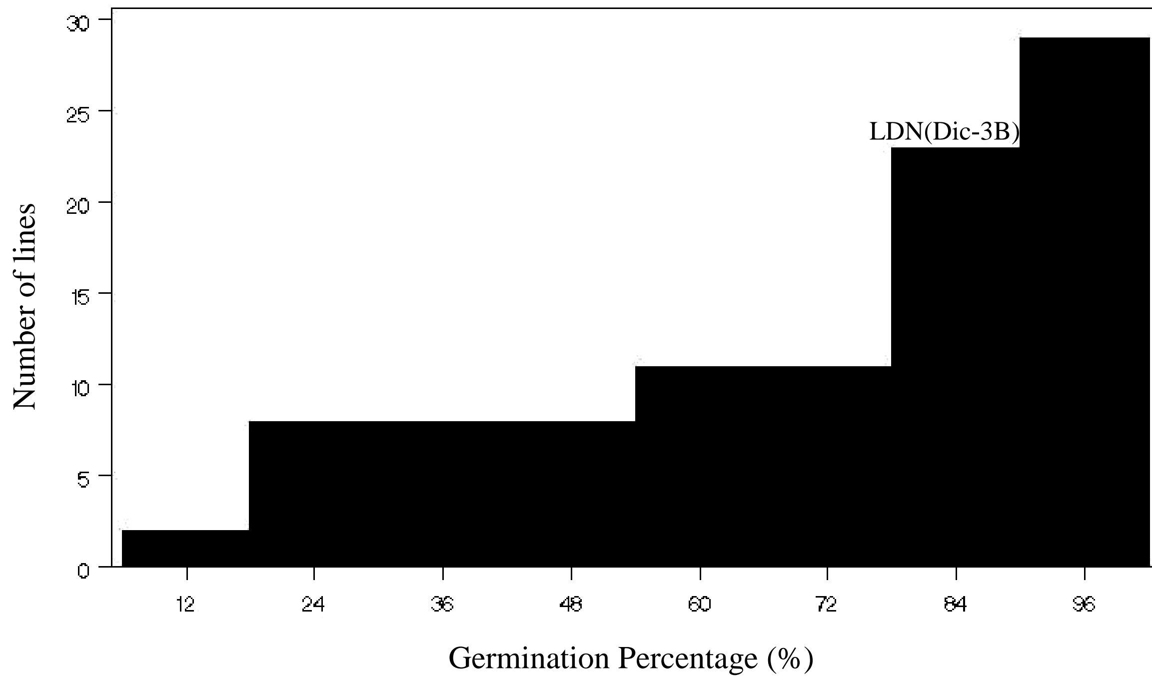


Figure 30. Frequency distribution of seed dormancy for LDN (Dic-3B) RICL population for Langdon 2006 at day 11 germination test.

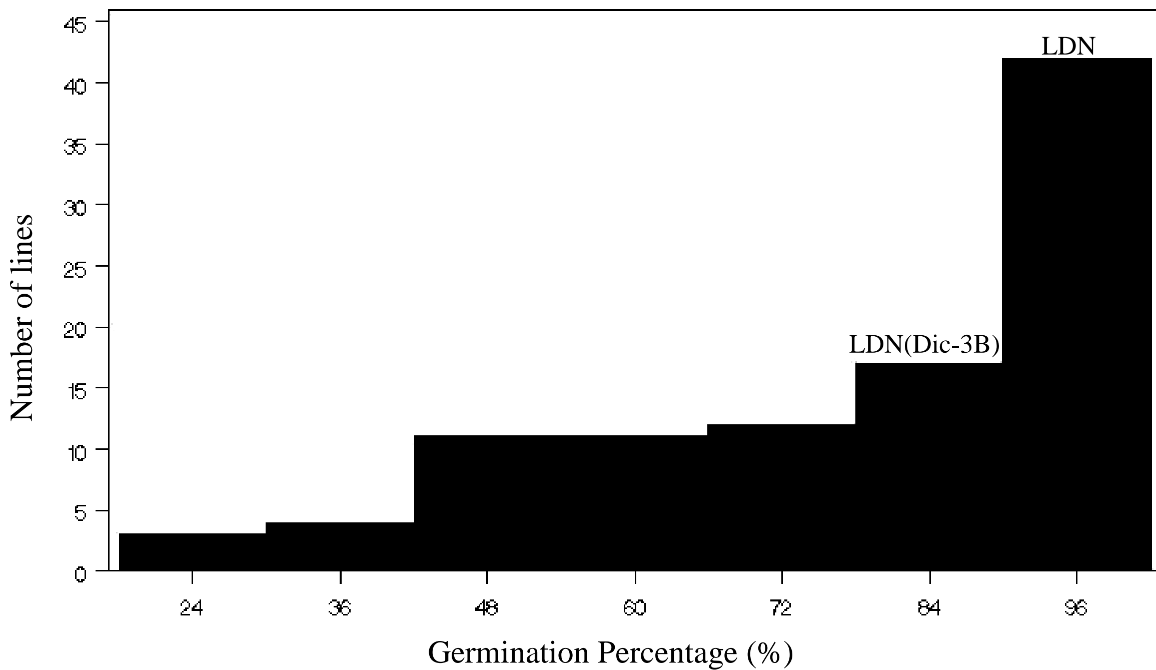


Figure 31. Frequency distribution of seed dormancy for LDN (Dic-3B) RICL population for Langdon 2006 at day 21 germination test.

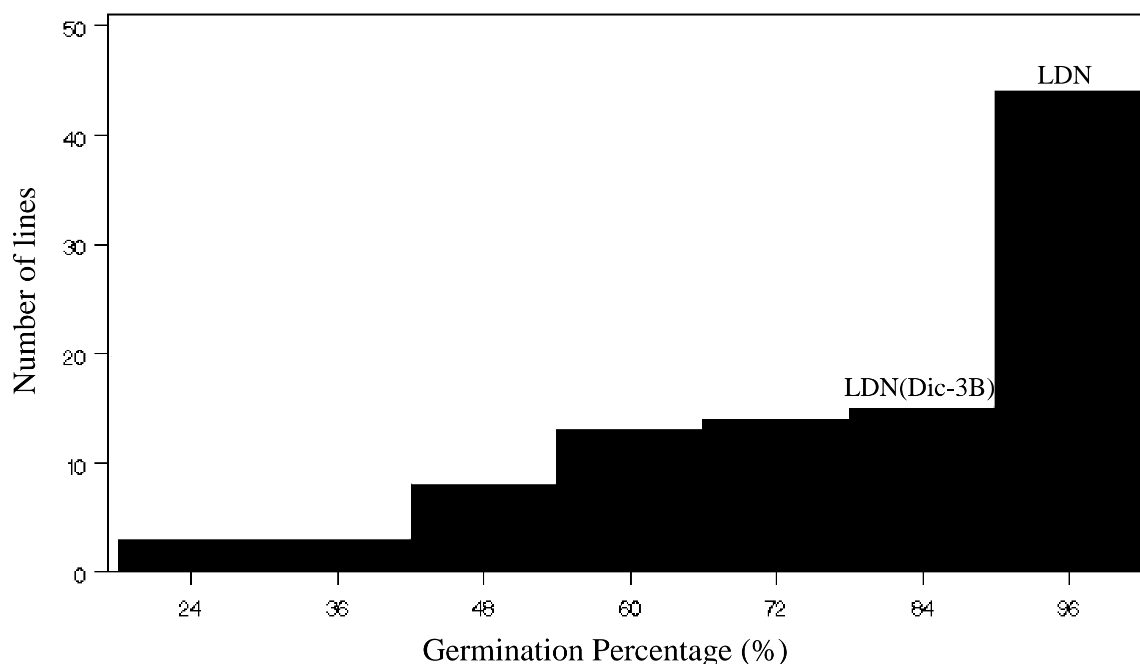


Figure 32. Frequency distribution of seed dormancy for LDN (Dic-3B) RICL population for Langdon 2006 at day 30 germination test.

Table17. Phenotypic means for percent germination of the parents, population and checks at Prosper and Langdon locations for year 2007

Genotype	Prosper 2007				Langdon 2007			
	Day1	Day11	Day21	Day30	Day1	Day11	Day21	Day30
LDN Dic 3B	26	44	46	46	5	38	44	48
Langdon	42	58	67	69	19	41	42	43
Population	18.2	35.7	42.2	43.3	24.4	43.4	50.6	52.8
Divide	22	53	61	63	3	9	12	12
Lebsock	15	44	48	48	20	31	39	39
Mountrail	11	20	23	23	11	27	31	32
Ben	36	66	69	69	20	31	31	31
Chahba88//B	12	40	44	45	6	18	25	28
RI4137	3	7	8	9	0	2	5	7
Iact12/Ka	28	59	60	60	19	31	33	34
LSD	16.8	23.9	26.5	27.6	17.8	25.1	29.5	31.2
CV	73.9	49.9	42.4	41.1	63.6	49.4	40.5	37.6

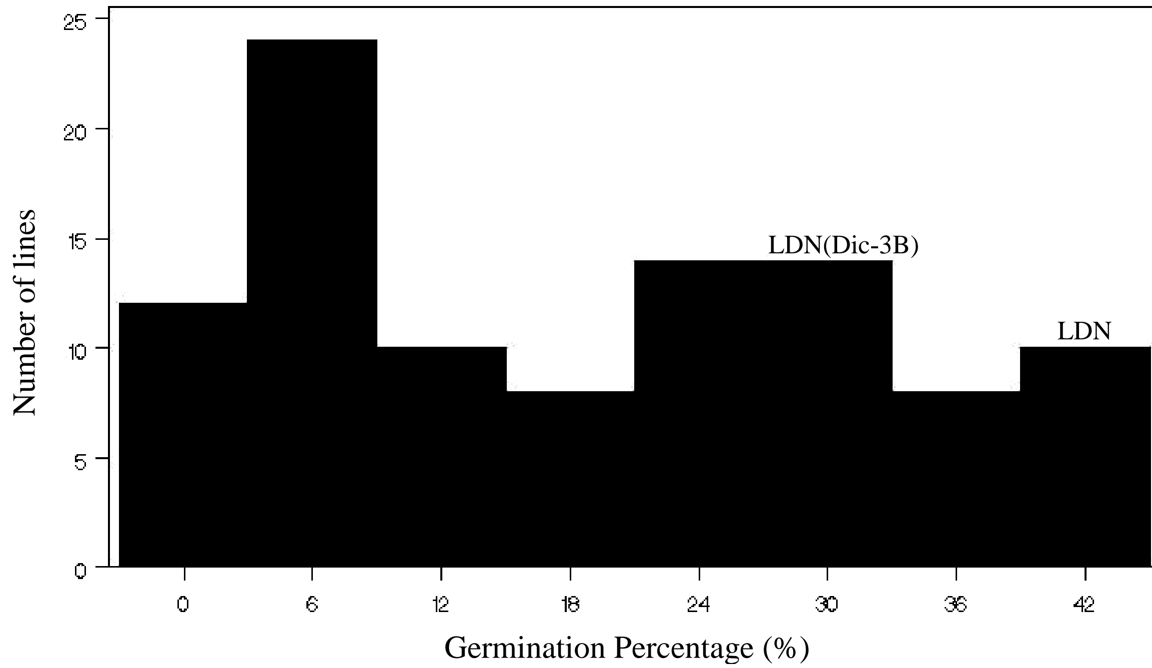


Figure 33. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Prosper 2007 at day 1 germination test.

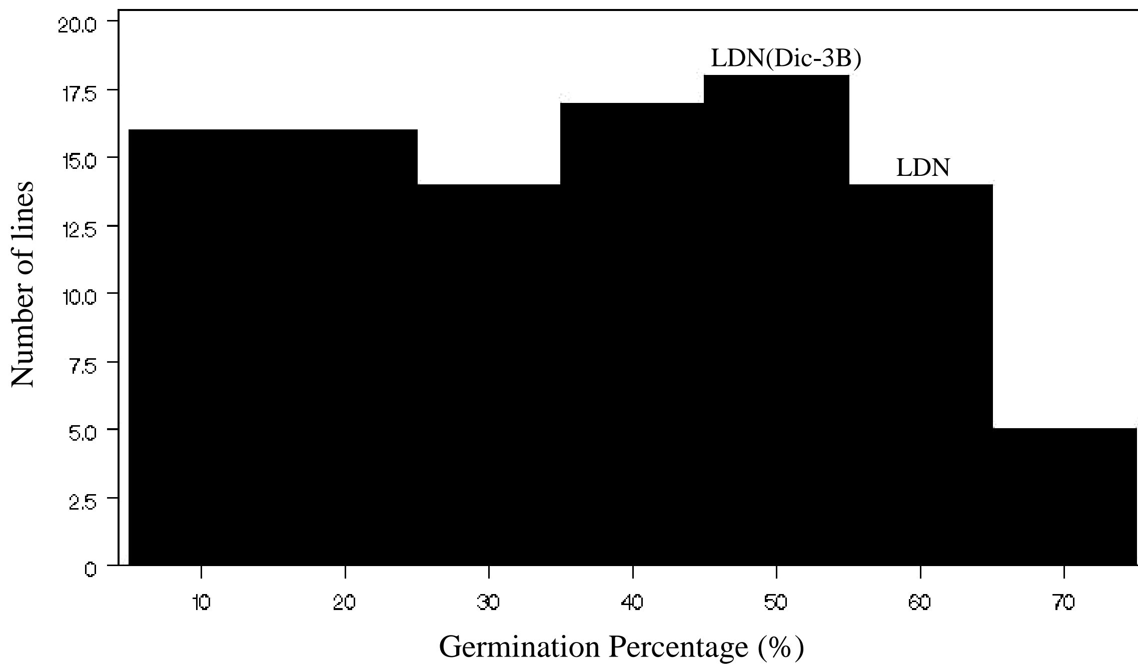


Figure 34. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Prosper 2007 at day 11 germination test.

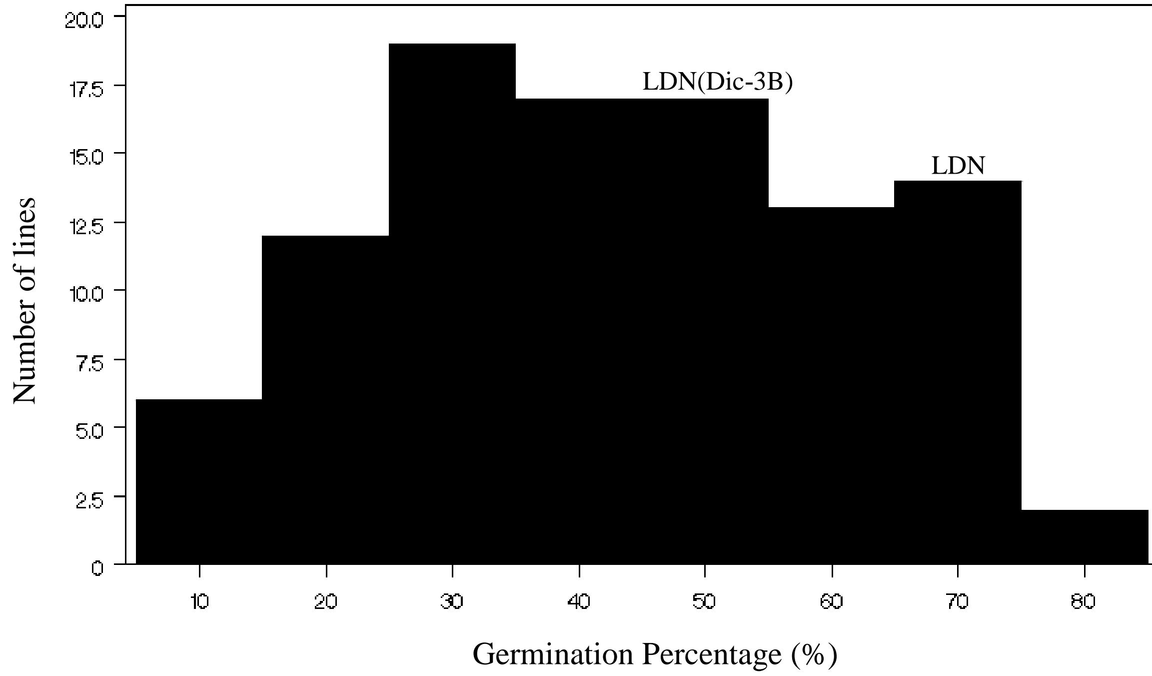


Figure 35. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Prosper 2007 at day 21 germination test.

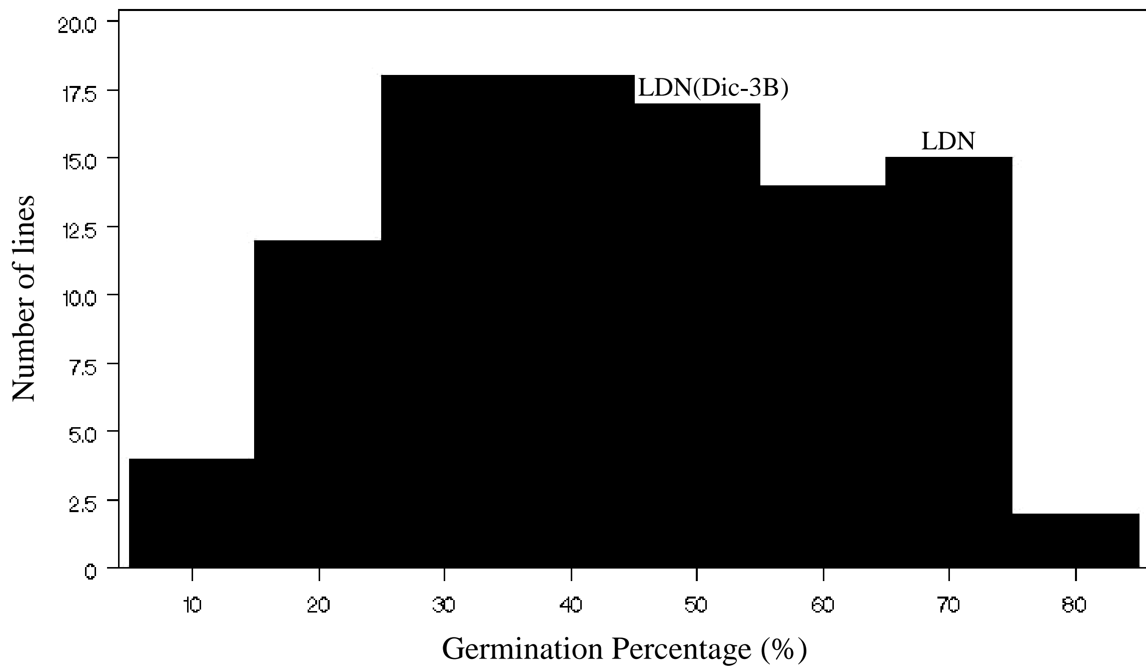


Figure 36. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Prosper 2007 at day 30 germination test.

Table 18. Shapiro-Wilk's normality test for seed dormancy for LDN (Dic 3B) RICL population for 2007 field locations

	W:Normal		Pr<W		Skewness	
	Prosper	Langdon	Prosper	Langdon	Prosper	Langdon
Day 1	0.91	0.94	0.0001	0.0005	0.27	0.2
Day 11	0.95	0.94	0.0027	0.0002	-0.02	0.02
Day 21	0.96	0.96	0.0121	0.0093	-0.006	0.03
Day 30	0.96	0.97	0.0186	0.0238	0.002	-0.002

The population, however, looked more like the dormant parent for the four days (Table 18 and Figure 37, 38, 39 and 40). Transgressive segregants were observed for both dormant and non dormant parents.

The genotypes for both Prosper and Langdon location for year 2007 were highly significant for the dormancy trait at all the four days, although the genotypic mean square values for day 11 was maximum (Appendix D Table D1, D2, D3 and D4). Thereby, indicating presence of large amount of variation in the population for the seed dormancy trait. The test for homogeneity of variance performed at day 1, 11, 21 and 30 for all the four field locations namely, Prosper 2006, Langdon 2006, Prosper 2007 and Langdon 2007, showed that seed dormancy observed at day 1 and 11 were heterogeneous over the locations, and thereby these locations could not be combined for these two days (Appendix E Table E1). However, for day 21 and 30 the seed dormancy trait was homogeneous across all the four locations (Appendix E Table E1). Thus, allowing combining the data for all the four locations. The combined analysis for day 21 and day 30 showed significant G x E interaction, thereby indicating that the environment has an effect on the genotypes for the seed dormancy trait (Appendix F Table F1).

As for the greenhouse material grown in Fall 2005, the parents were significantly different from each other for the dormancy trait for day 11, 21 and 30 (Table 19) and the genotypes were found to be non significant (Appendix D Table D5).

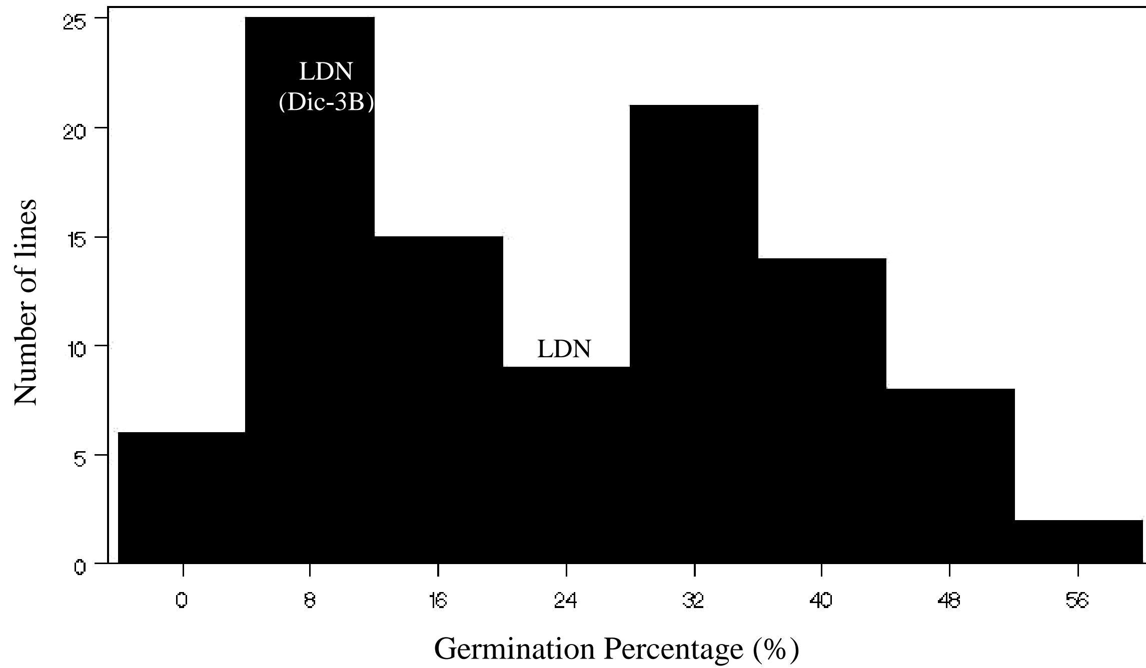


Figure 37. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Langdon 2007 at day 1 germination test.

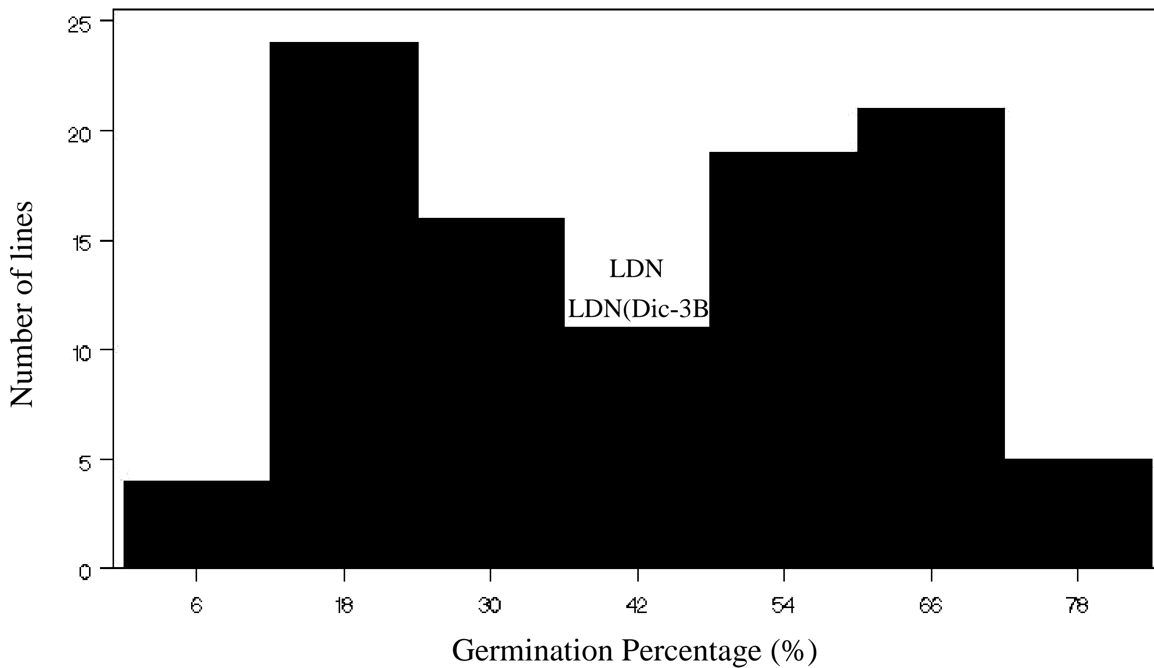


Figure 38. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Langdon 2007 at day 11 germination test.

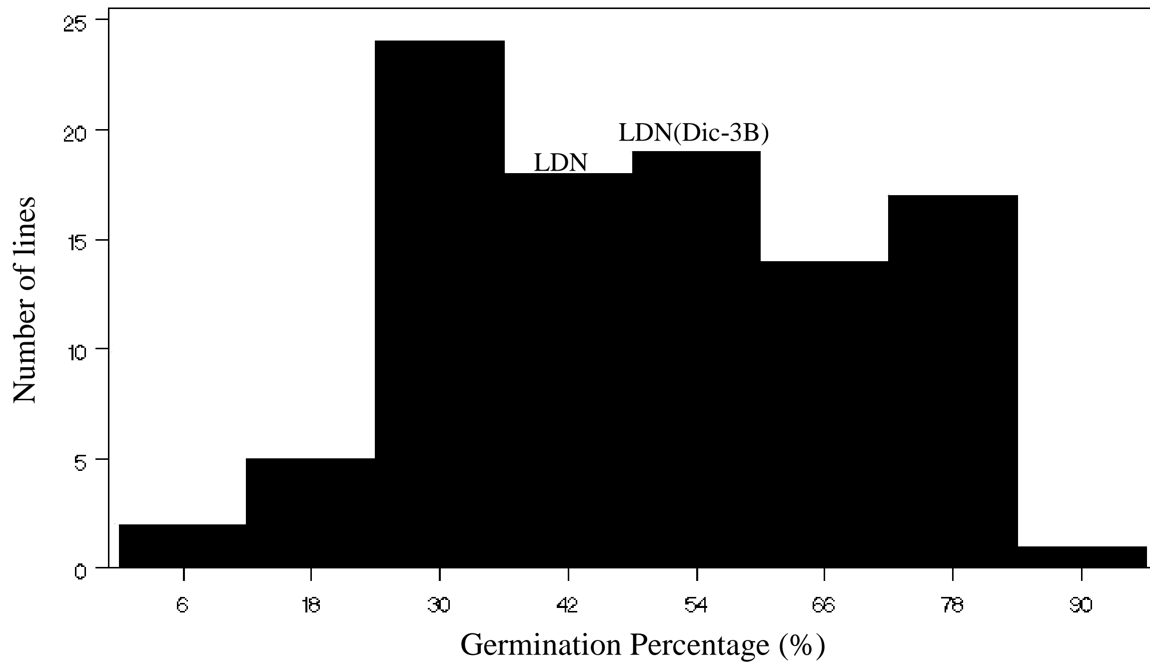


Figure 39. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Langdon 2007 at day 21 germination test.

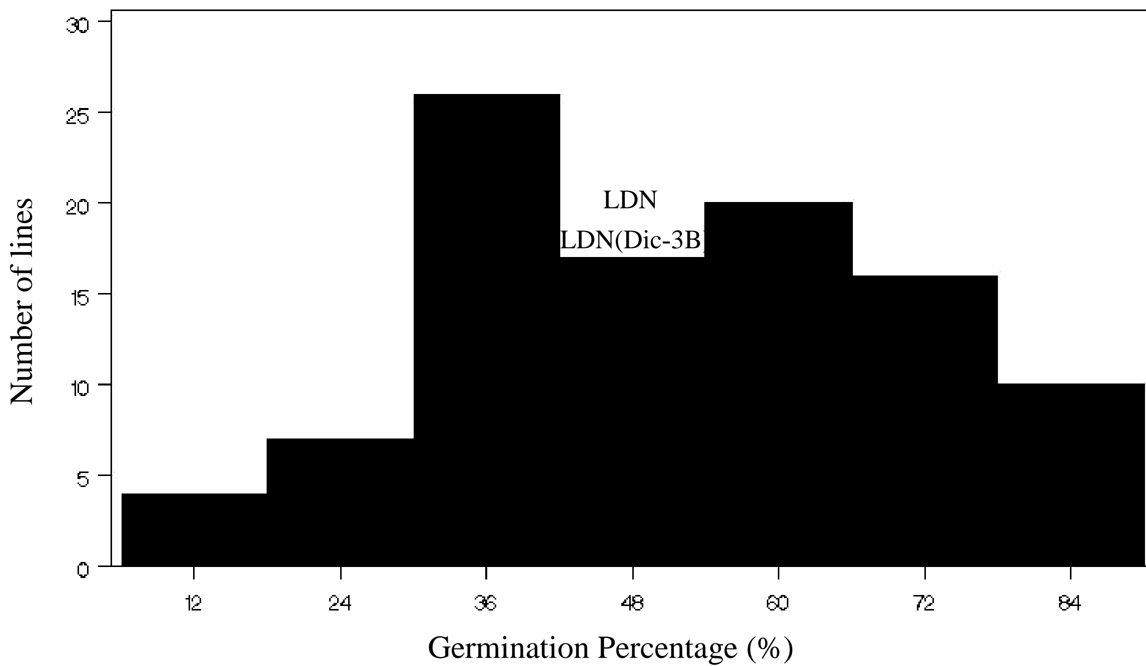


Figure 40. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Langdon 2007 at day 30 germination test.

Table 19. Phenotypic means for percent germination of the parents and population for Fall greenhouse season germinated at 20C

Genotypes	Fall			
	Day 1	Day 11	Day 21	Day 30
LDN Dic 3B	0	0	4.6	14.6
LDN	0	24	34.6	41.3
Population	0	4.2	10.2	15
LSD	-	9.8	11.1	15.3
CV	-	95.6	62.1	50.8

The Spring 2006 material tested for dormancy at 20 and 27C, population tested at both 20 and 27C showed significant differences for seed dormancy trait at day 11, 21 and 30 (Appendix D Table D6 and D7). The genotypes were non significant at day 1 for the population tested at 27C, whereas it was significant for the material tested for dormancy at 20C (Appendix D Table D6 and D7). The day 11 samples showed maximum value for the genotypic mean squares for the plant material tested at both 20 and 27C for seed dormancy. The Spring 2006 material tested at 27C had larger genotypic mean square values as compared to the material tested at 20C (Appendix D Table D6 and D7).

The Levene's test for homogeneity of variance for the two greenhouse seasons suggested that they were heterogeneous for the seed dormancy trait for day 1, 11 21 and 30, when tested at 20C and for day 11, 21 and 30, when tested at 27C (Appendix E Table E2 and E3). However, the two seasons were homogeneous for seed dormancy observed at day 1 when tested at 27C (Appendix E Table E3). The two seasons were combined for day 1 and did not show any significant GxE interaction (Appendix F Table F2). Thereby, indicating that even with the change in the season there was no effect on the genotype on day 1 for seed dormancy trait when tested at 27C. The frequency distribution pattern indicated that the population was skewed towards the dormant parent for Fall season and towards non dormant parent for Spring season (Table 21).

Table 20. Phenotypic means for percent germination of the parents and population for Spring greenhouse season germinated at 20C and 27C

Genotypes	Spring 20C				Spring 27C			
	Day1	Day11	Day21	Day30	Day1	Day11	Day21	Day30
LDN Dic-3B	0	81.3	93.3	93.3	0	65.3	85.3	89.3
LDN	1.3	90.6	97.3	97.3	0	85.3	98.6	100
Population	1.4	60.1	75.1	80	0.1	42.9	64.9	72.4
LSD	4.2	18.3	15.5	14	-	15.3	15.0	16
CV	175.7	40.7	29.1	24.8	380.6	61	40.9	32

Table 21. Shapiro-Wilk's normality test for seed dormancy for LDN(Dic-3B) RICLs for Fall (2005) and Spring (2006) greenhouse seasons

	W:Normal			Pr<W			Skewness		
	Spring 27C	Spring 20C	Fall	Spring 27C	Spring 20C	Fall	Spring 27C	Spring 20C	Fall
Day1	0.26	0.6	-	0.0001	0.0001	-	3.58	3.26	-
Day11	0.95	0.93	0.82	0.0021	0.0001	0.0001	0.08	-0.34	1.81
Day21	0.9	0.87	0.95	0.0001	0.0001	0.0021	-0.54	-0.86	0.79
Day30	0.88	0.82	0.96	0.0001	0.0001	0.0113	-0.73	-1.15	0.68

Transgressive segregants for dormancy was observed for Spring season material tested for dormancy at both 20 and 27C (Figure 41 to 50). This could be attributed to the presence of large number of genes present in the population that are contributing positively towards the dormancy trait.

Frequency distribution patterns were also studied for plant height and days to flowering traits recorded for Prosper and Langdon locations for years 2006 and 2007 each. Days to flowering for Prosper 2006 location showed parents to be significantly different from each other (Table 22). Many transgressive segregants were observed for the non-dormant parent, Langdon-16, that was late maturing type (Figure 51). Similarly, for Langdon 2006 location the parents were significantly different (Table 22) and population was more skewed towards Langdon-16 (Table 23 and Figure 52). The difference between parents for the days to flowering trait for Langdon 2007 location was large (Table 24). The population spanned between LDN (Dic-3B) and LDN parents for the days to flowering trait for the Langdon 2007 location. Very few transgressive segregants were observed for either of the parents (Figure 53).

The genotypes were significantly different for days to flowering for all the field locations (Appendix D Table D8). The test for homogeneity of variance revealed that all the locations were homogeneous for this trait (Appendix E Table E4), thereby allowing us to combine the data over the locations. The combined analysis revealed significant GxE interaction (Appendix F Table F3). Thus suggesting that environment has an effect on the genotype for the days to flowering trait.

For the plant height trait Langdon 16 was found to be taller than LDN (Dic-3B) for all the four field locations. However, the significant difference between the parents for this trait was only observed for Prosper 2006 location (Table 22 and 24). The population was negatively skewed for all the four field locations namely, Prosper 2006, Langdon 2006, Prosper 2007 and Langdon 2007 location (Table 23 and 25 and Figure 54, 55, 56 and 57). Thereby indicating, the population was similar to Langdon 16 that is the tall parent.

The genotypes were significantly different for the plant height for all the four field locations (Appendix D Table D9). The test for homogeneity of variance suggested that all locations were homogeneous and thereby, they could be combined for the plant height trait (Appendix E Table E4). The combined analysis did not show any GxE interaction (Appendix F Table F4). Thereby indicating that environment had no effect on the plant height trait at these four different locations.

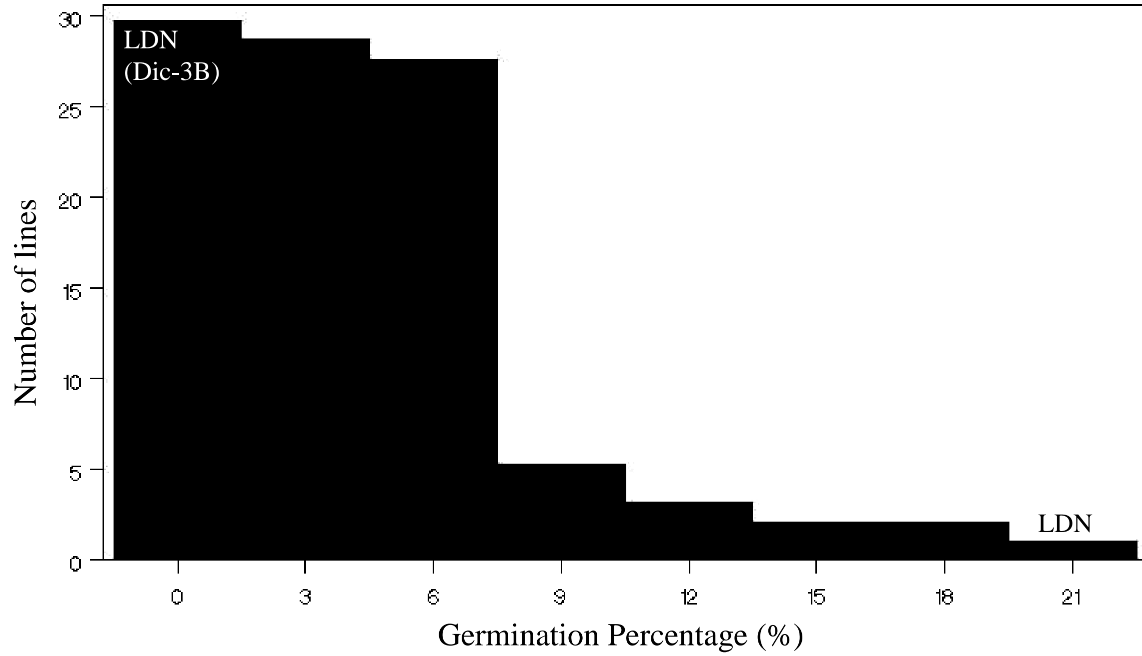


Figure 41. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Fall 2005 at day 11 germination test at 20^{0}C.

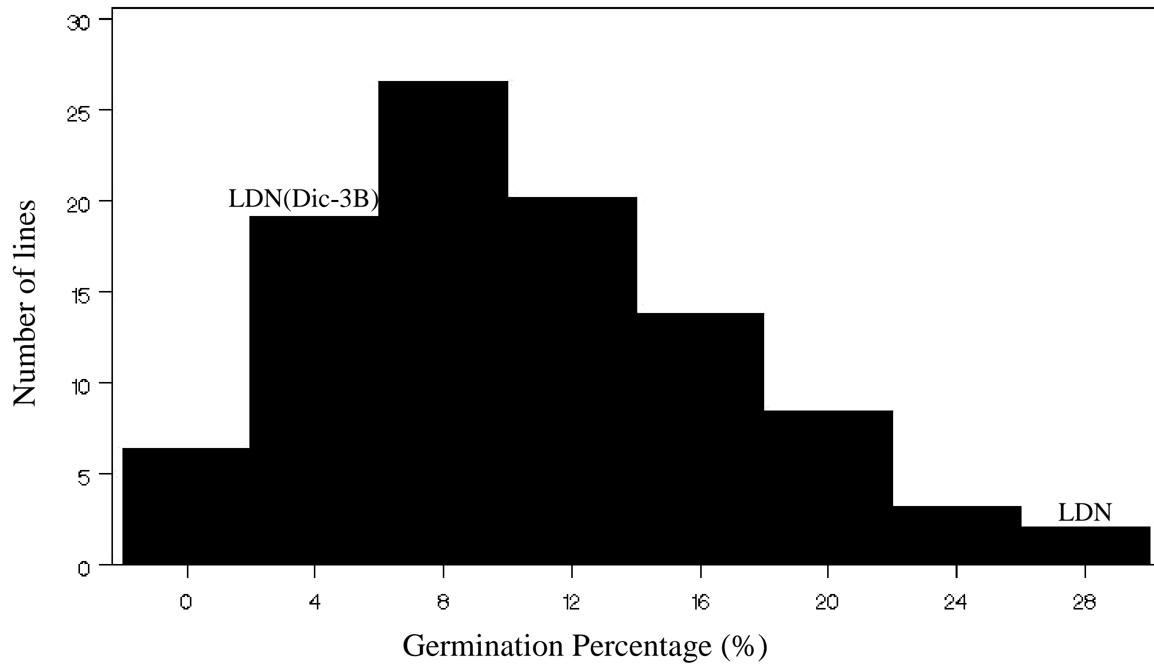


Figure 42. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Fall 2005 at day 21 germination test at 20^{0}C.

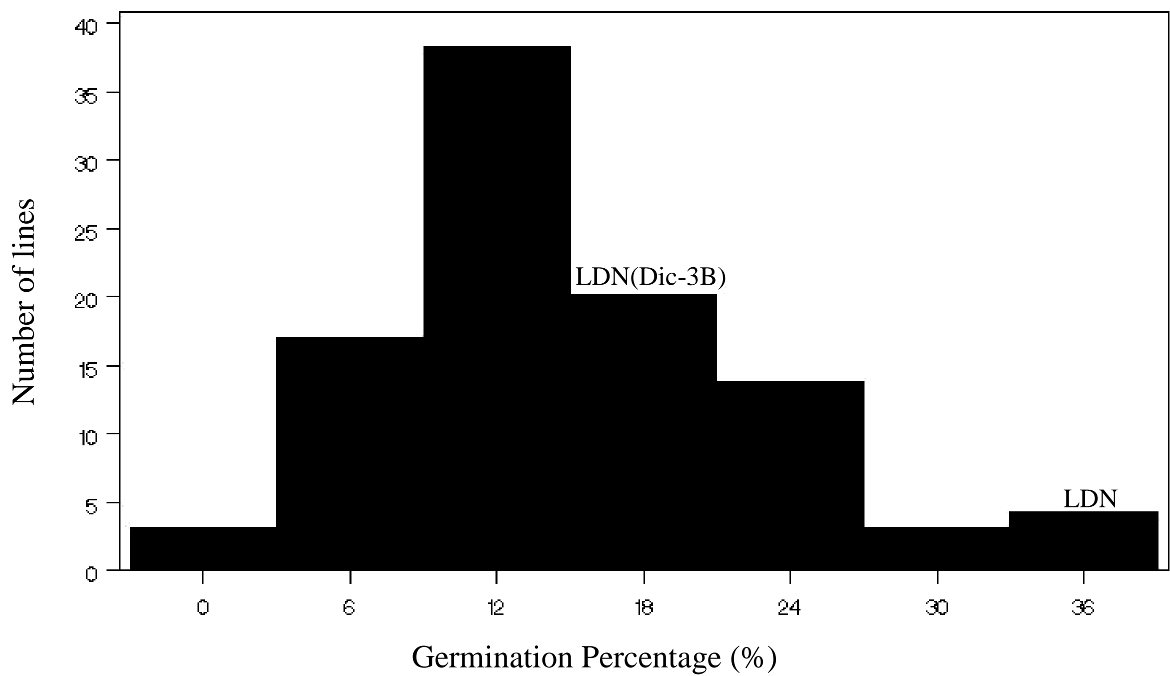


Figure 43. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Fall 2005 at day 30 germination test at 20^0C.

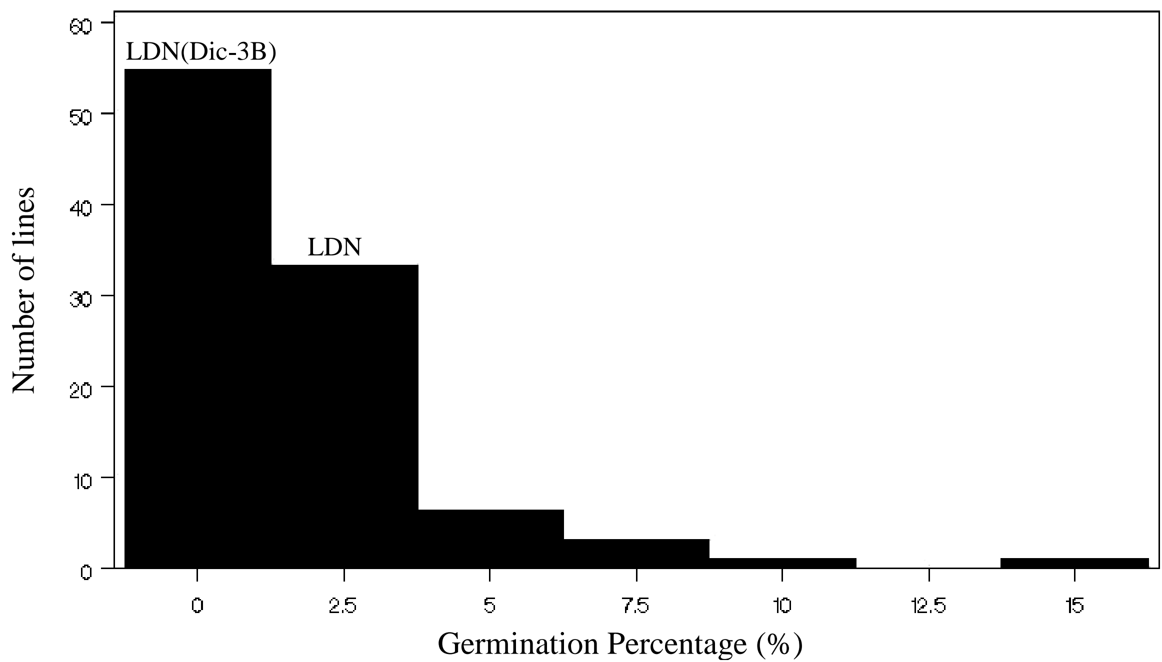


Figure 44. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Spring 2005 at day 1 germination test at 20^0C.

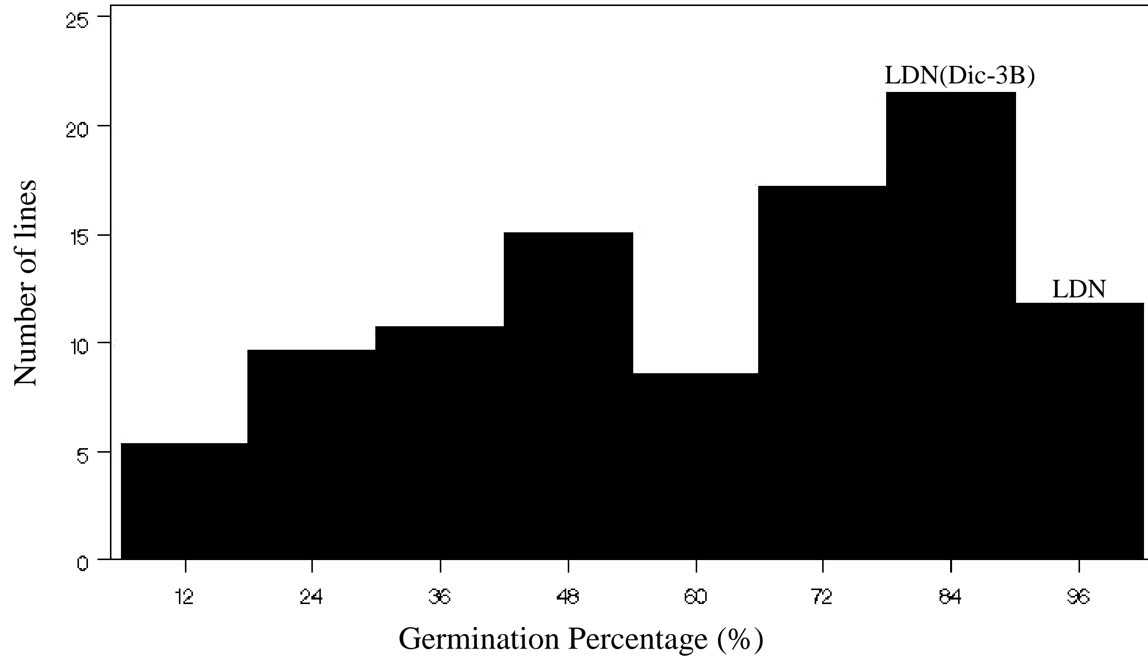


Figure 45. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Spring 2005 at day 11 germination test at 20^0C.

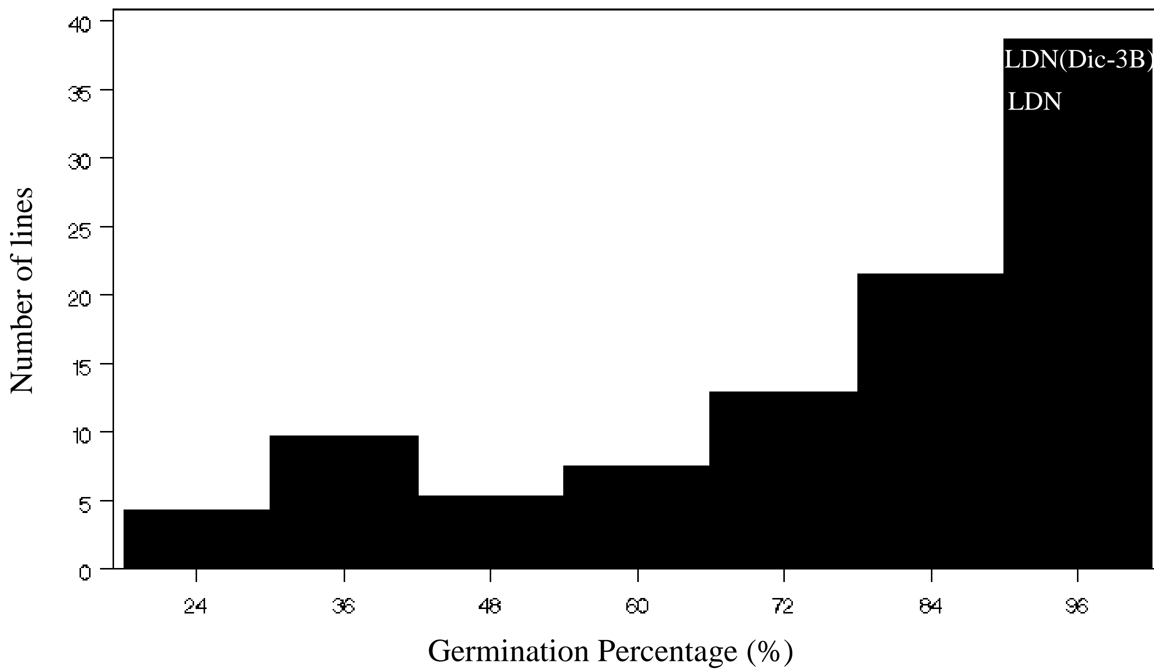


Figure 46. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Spring 2005 at day 21 germination test at 20^0C.

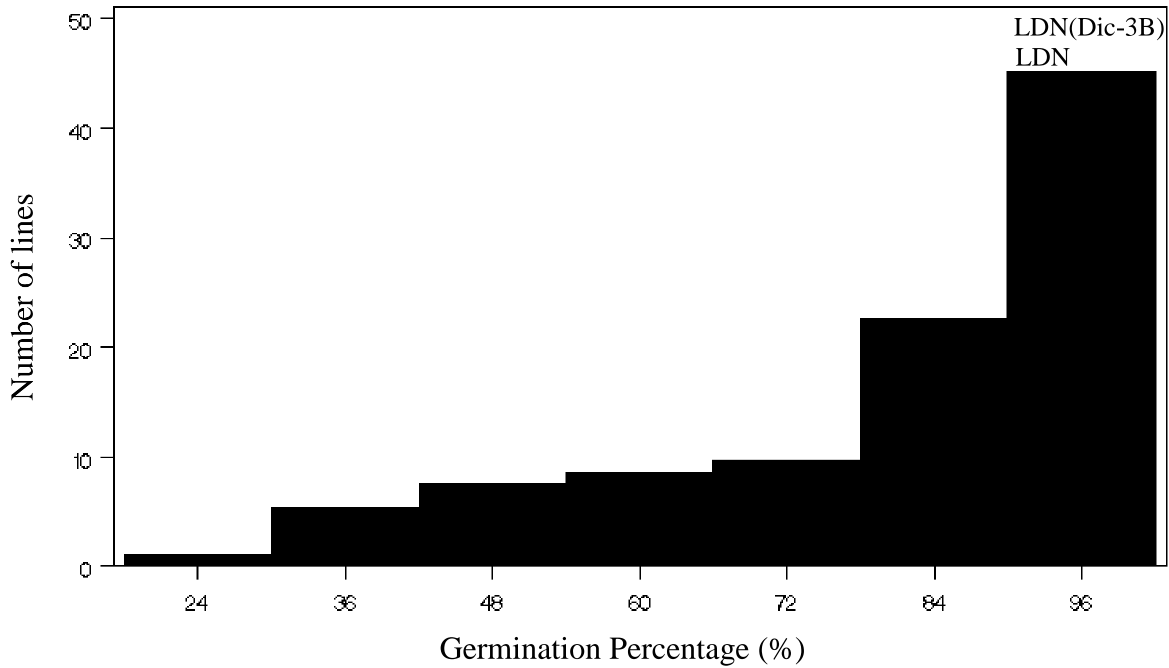


Figure 47. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Spring 2005 at day 30 germination test at 20^{0}C.

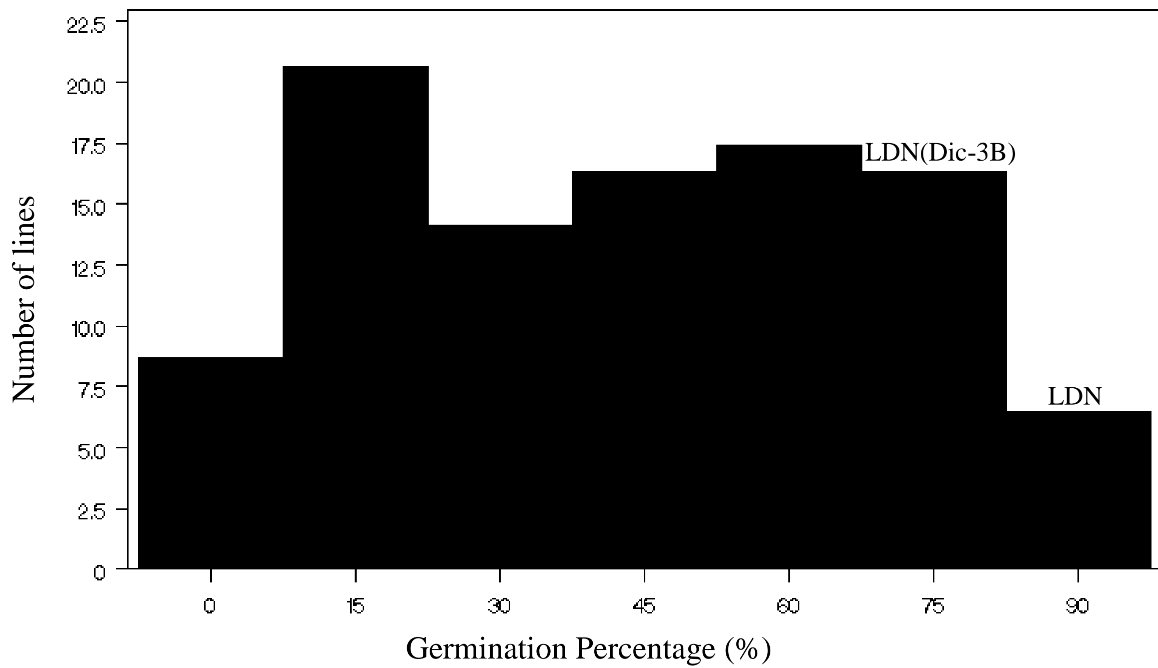


Figure 48. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Spring 2005 at day 11 germination test at 27^{0}C.

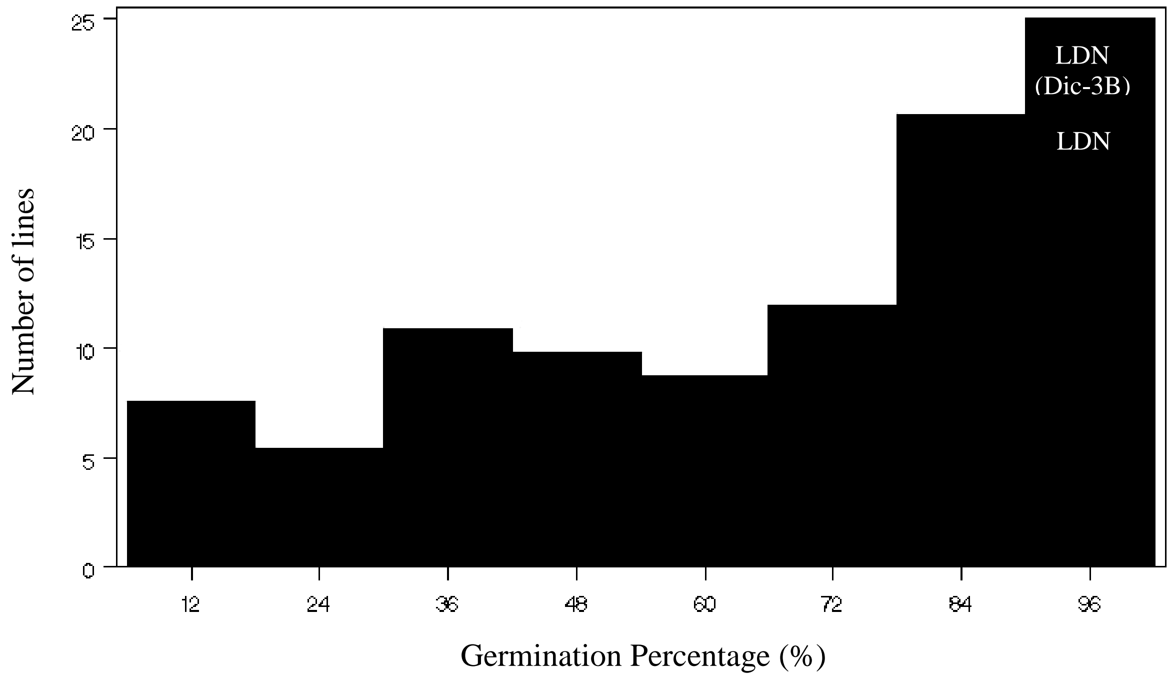


Figure 49. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Spring 2005 at day 21 germination test at 27^0C.

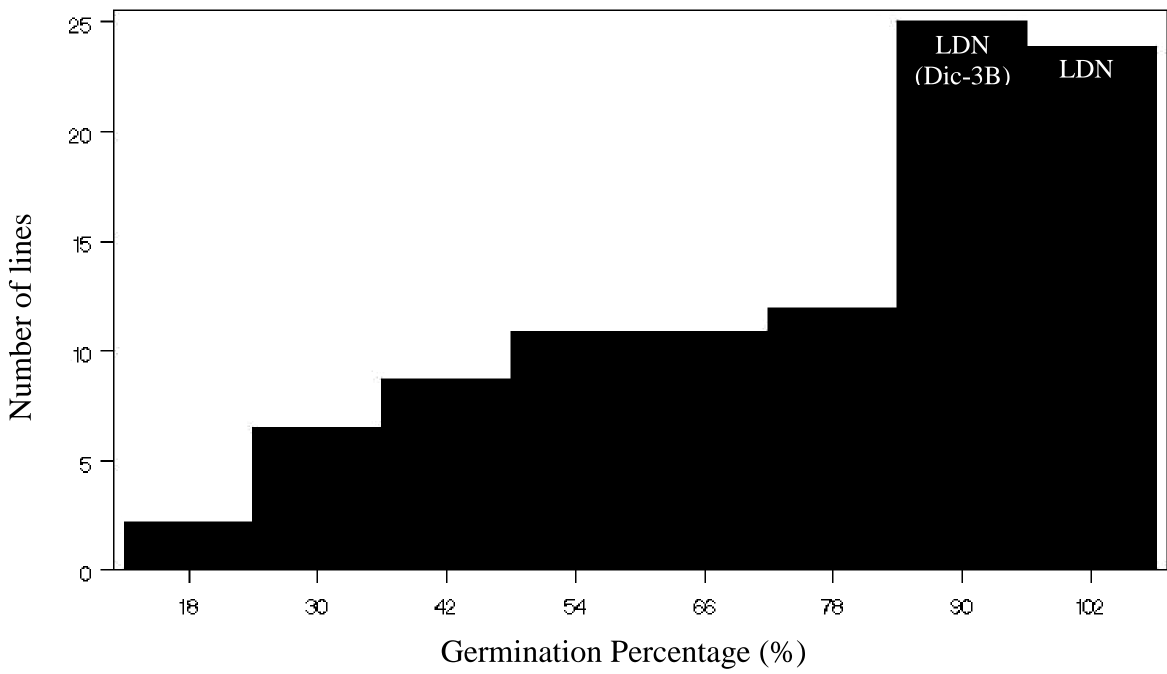


Figure 50. Frequency distribution of seed dormancy for LDN (Dic 3B) RICL population for Spring 2005 at day 30 germination test at 27^0C.

Table 22. Phenotypic means for days to flowering and plant height of the parents, population and checks at Prosper and Langdon locations for year 2006

Genotype	Prosper 2006		Langdon 2006	
	DOF	HT	DOF	HT
LDN Dic 3B	60	100	58.5	112.5
Langdon	64	128	61	120
Population	63.12	121.48	62.09	120.43
Divide	60	92.5	59	110
Lebsock	59.5	90	56	96
Mountrail	60	87	58.5	100
Ben	60	91.5	57.5	97
Chahba88//B	59.5	75	55.5	99
RI4137	61	95	55	104
Iact12/Ka	58.5	84	54.5	90
LSD	2.29	11.92	1.89	16.18
CV	4.06	10.37	4.8	7.4

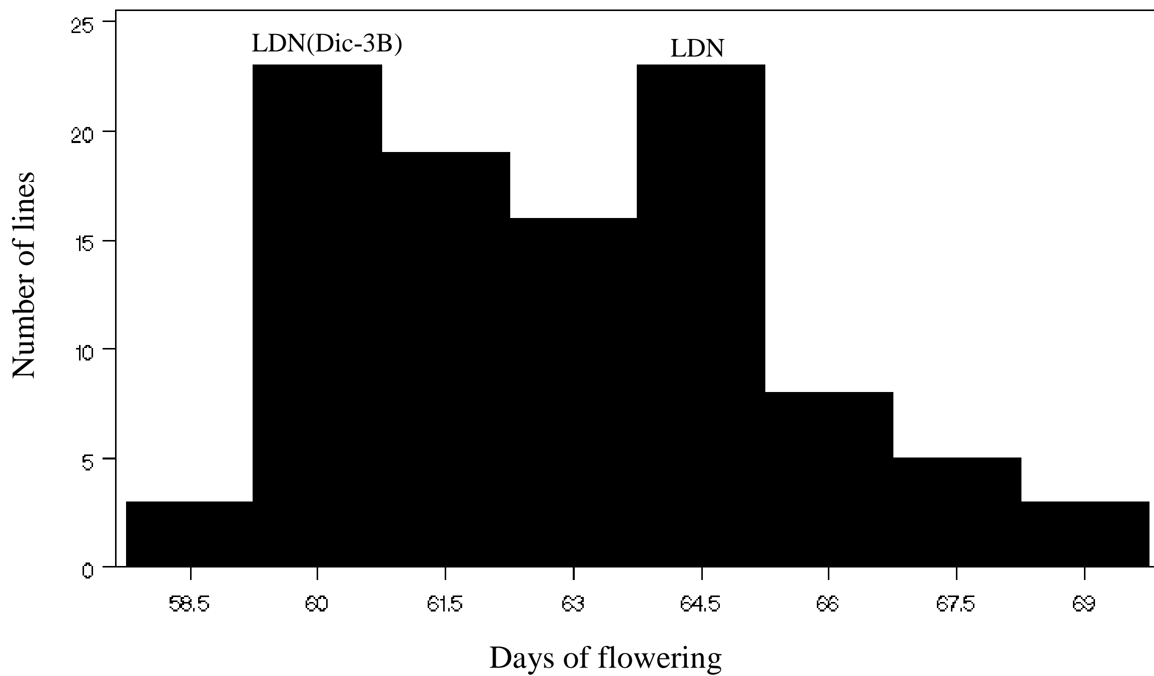


Figure 51. Frequency distribution of days of flowering for LDN (Dic 3B) RICL population for Prosper 2006.

Table 23. Shapiro-Wilk's normality test for agronomic traits for LDN (Dic 3B) RICLs for 2006 field locations

	W:Normal		Pr<W		Skewness	
	Prosper	Langdon	Prosper	Langdon	Prosper	Langdon
Days to Flowering	0.95	0.92	0.0035	0.0001	0.41	-0.33
Plant Height	0.91	0.95	0.0001	0.0025	-1.2	-0.8

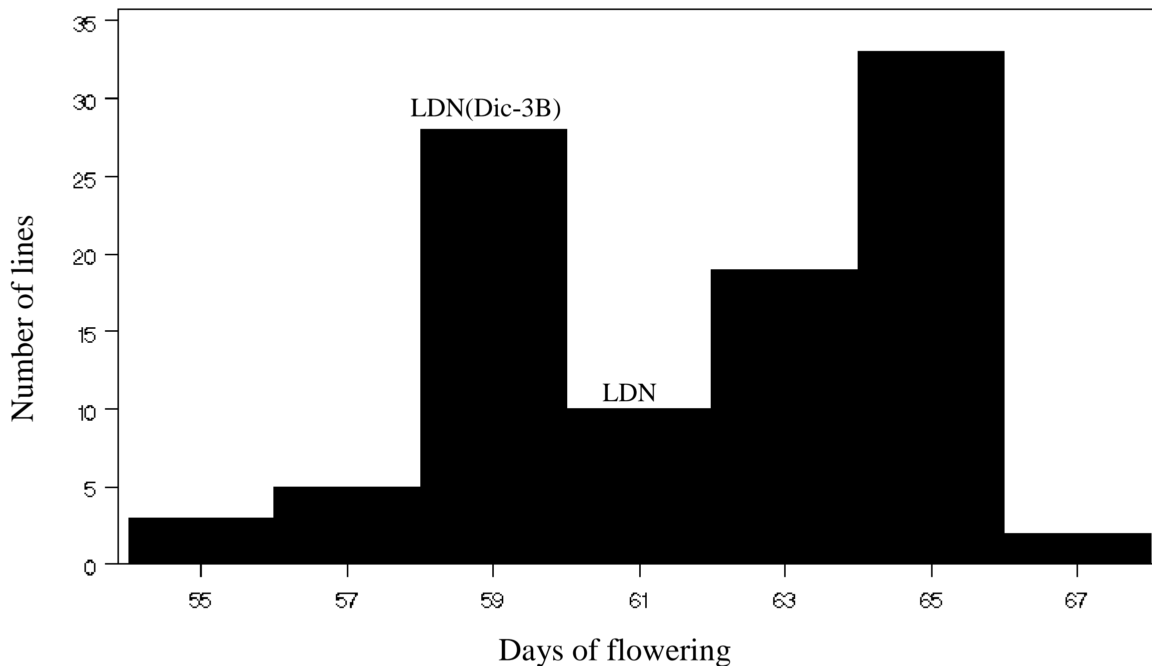


Figure 52. Frequency distribution of days of flowering for LDN (Dic 3B) RICL population for Langdon 2006.

Table 24. Phenotypic means for days to flowering and plant height of the parents, population and checks at Prosper and Langdon locations for year 2007

Genotype	Prosper 2007	Langdon 2007	
	HT	DOF	HT
LDN Dic 3B	102.5	62.5	120
Langdon	107.5	70	124
Population	103.35	66.59	127.46
Divide	86.5	65	102.5
Lebsock	82.5	63	102.5
Mountrail	85	65	107.5
Ben	91	64.5	107.5
Chahba88//B	77.5	60.5	79.5
RI4137	82.5	64	105
Iact12/Ka	77.5	60	98.5
LSD	17.24	2.89	13.08
CV	8.4	4.6	7.12

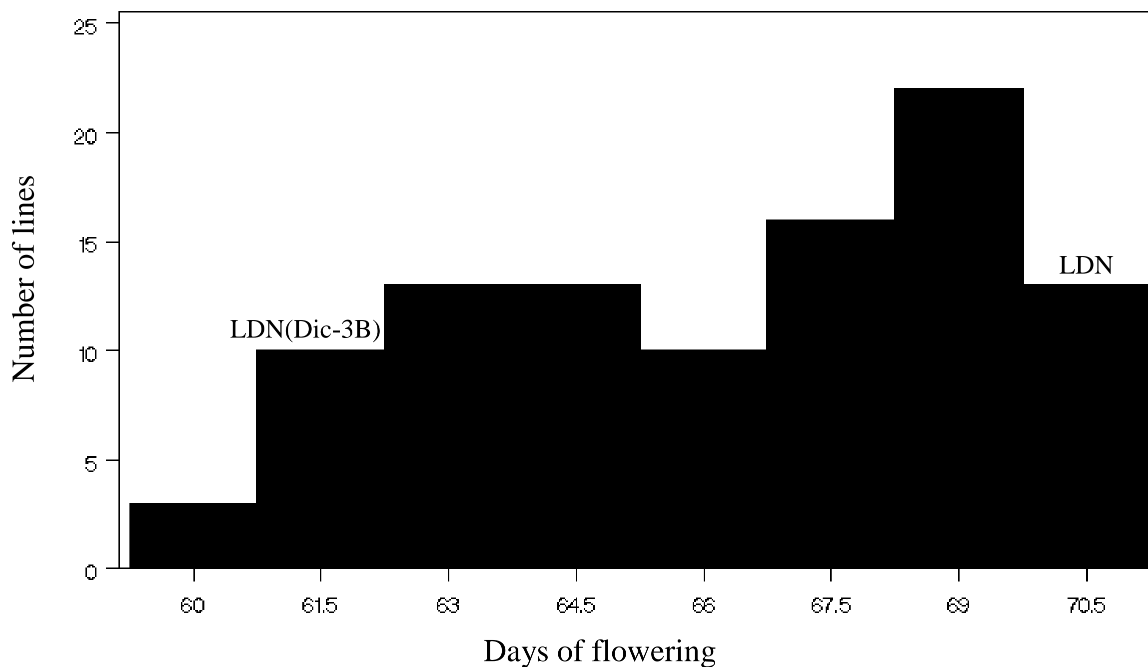


Figure 53. Frequency distribution of days of flowering for LDN (Dic-3B) RICL population for Langdon 2007.

Table 25. Shapiro-Wilk's normality test for agronomic traits for LDN(Dic-3B) RICLs at field locations (2007)

	W:Normal		Pr<W		Skewness	
	Prosper	Langdon	Prosper	Langdon	Prosper	Langdon
Days to Flowering	-	0.92	-	0.0001	-	-0.35
Plant Height	0.95	0.81	0.0034	0.0001	-0.69	-2.16

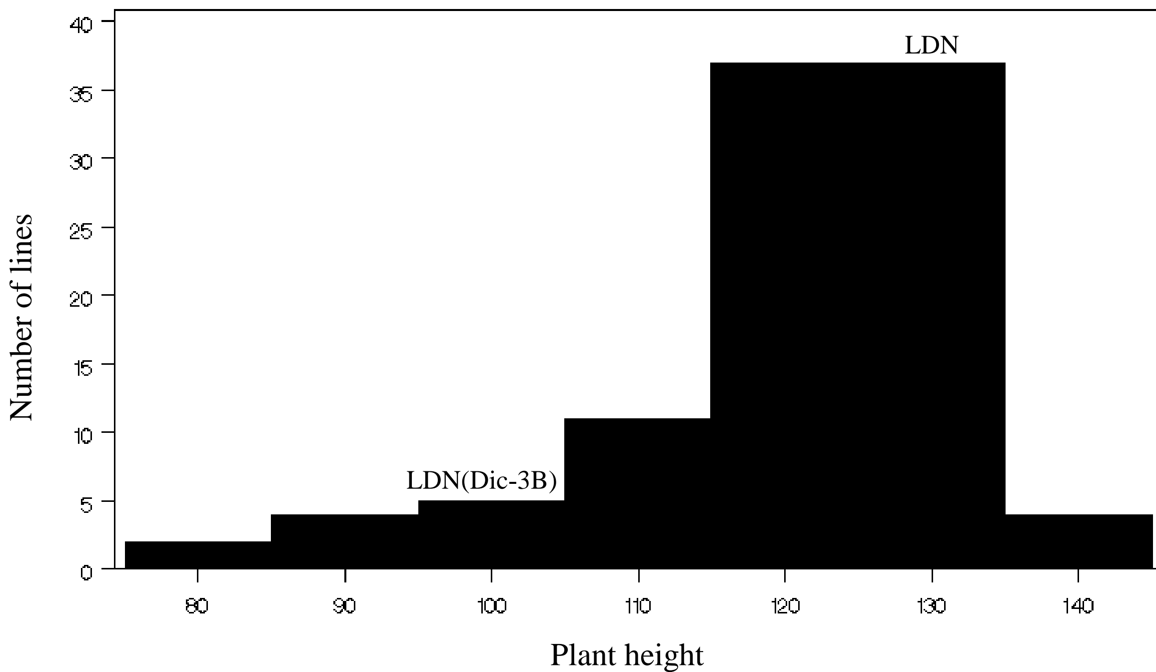


Figure 54. Frequency distribution of plant height for LDN (Dic-3B) RICL population for Prosper 2006.

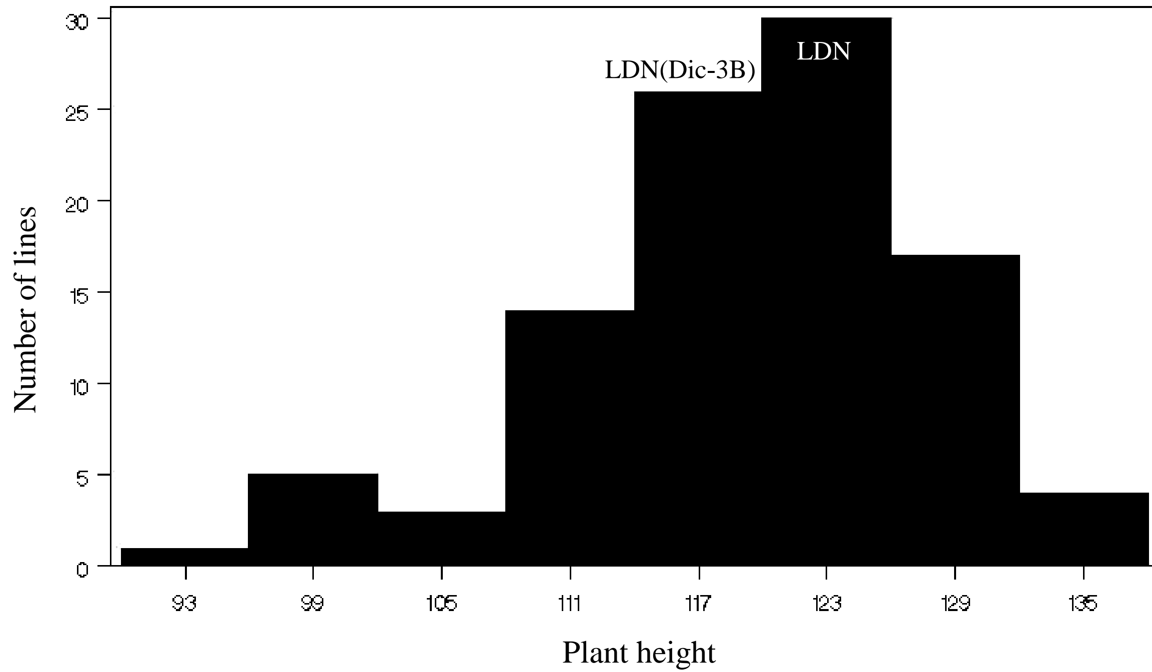


Figure 55. Frequency distribution of plant height for LDN (Dic-3B) RICL population for Langdon 2006.

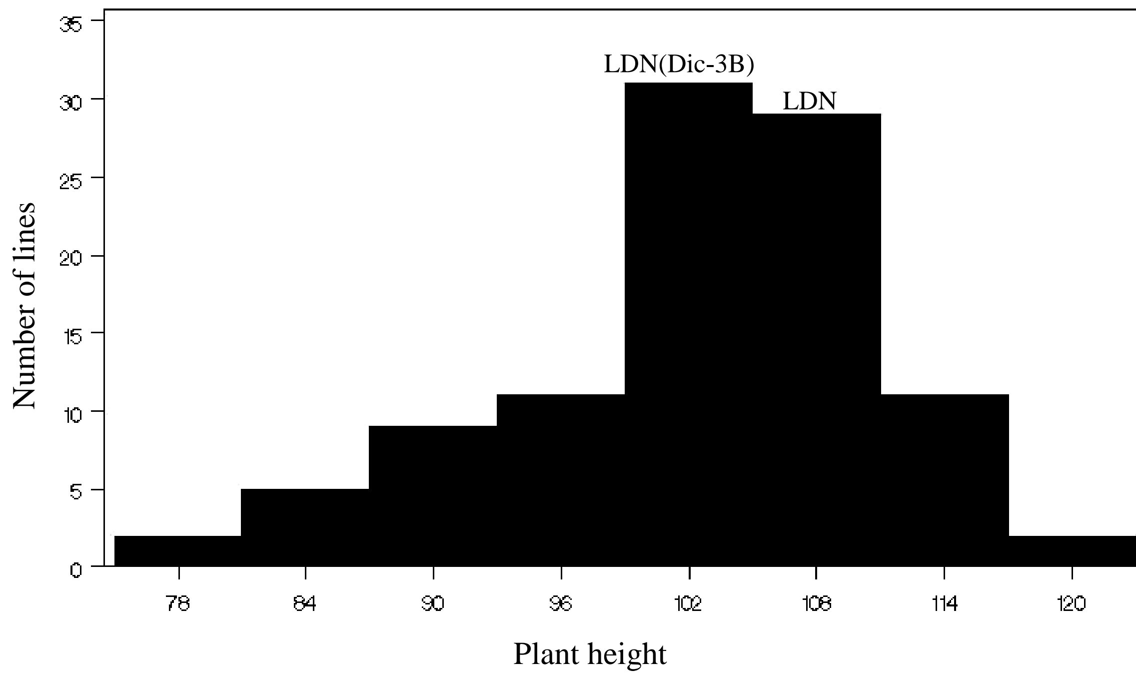


Figure 56. Frequency distribution of plant height for LDN (Dic-3B) RICL population for Prosper 2007.

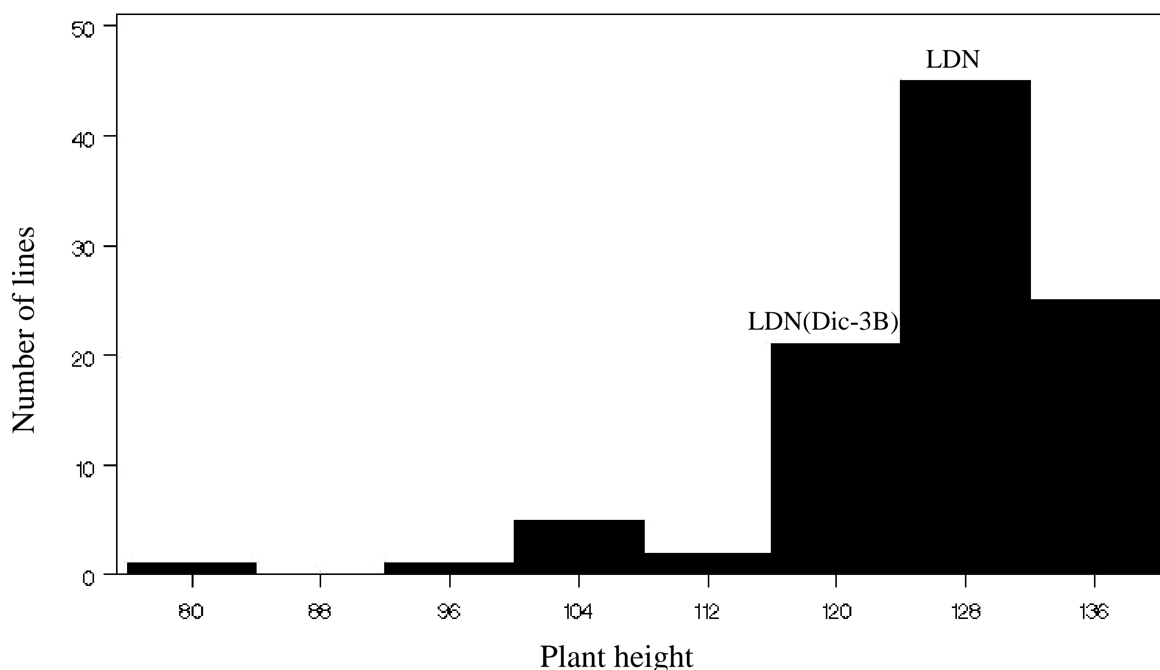


Figure 57. Frequency distribution of plant height for LDN (Dic-3B) RICL population for Langdon 2007.

Phenotypic correlations for seed dormancy and other agronomic traits on chromosome 3B

Phenotypic correlations were calculated for all the four field locations for seed dormancy, plant height and days to heading traits (Table 26). Days to heading was found to be negatively correlated to seed dormancy trait on day 11, 21 and 30 for prosper 2006 location and at day 1 for langdon2006 location. Days to heading was also correlated positively with plant height trait for all the field locations. Plant height was found to be positively correlated with seed dormancy trait at day 1, 11, 21 and 30 at Langdon 2007 location. Correlation was also studied for seed dormancy trait between 1, 11, 21 and 30 days to germination (Table 27 and Table 28). All the days were found to be highly correlated. Day 1 was found to be significantly correlated with day 11 and day 11 showed highest correlations with day 21 for all the field locations. Similarly day 21 was significantly correlated with day 30 for all the field locations.

Marker analysis for seed dormancy on chromosome 3B

To create the genetic linkage map of the chromosome group 3B, 58 microsatellites were screened for polymorphism on the two parents namely, LDN (Dic 3B) and LDN. Out of which, thirteen SSR's were polymorphic for the parents and were thus selected to screen the entire RICL population. Ten markers were mapped on the long arm of chromosome 3B. Two markers were mapped on different group and were therefore unlinked.

Table 26. Correlation coefficients for days to heading (DTH), plant height (HT) and seed dormancy recorded at day1, 11, 21 and 30 for the LDN (Dic 3B) RICL population

	Prosper 2006		Langdon 2006		Prosper 2007	Langdon 2007	
Days to gemination	DTH	HT	DTH	HT	HT	DTH	HT
Day1	-0.19	-0.06	-0.19*	-0.02	0.02	-0.08	0.22*
Day11	-0.28**	-0.10	-0.08	-0.01	-0.09	-0.12	0.24**
Day21	-0.27**	-0.05	-0.08	0.04	-0.06	-0.15	0.29**
Day30	-0.26**	0.02	-0.09	0.06	-0.06	-0.13	0.31**
DTH	-	0.59**	-	0.36**	-	-	0.27**

* Significant at ≤5% level of significance
** Significant at ≤1% level of significance

Table 27. Correlation coefficients for seed dormancy recorded at day 1, 11, 21 and 30 for the LDN (Dic 3B) RICL population for field locations for year 2006

	Prosper 2006				Langdon 2006			
	Day 1	Day 11	Day 21	Day 30	Day 1	Day 11	Day 21	Day 30
Day 1	1.00	0.75**	0.60**	0.57**	1.00	0.81**	0.79**	0.77**
Day 11	-	1.00	0.92**	0.84**	-	1.00	0.97**	0.96**
Day 21	-	-	1.00	0.95**	-	-	1.00	0.99**
Day 30	-	-	-	1.00	-	-	-	1.00

** = highly significant at ≤1% level of significance

Table 28. Correlation coefficients for seed dormancy recorded at day 1, 11, 21 and 30 for the LDN (Dic 3B) RICL population for field locations for year 2007

	Prosper 2007				Langdon 2007			
	Day 1	Day 11	Day 21	Day 30	Day 1	Day 11	Day 21	Day 30
Day 1	1.00	0.90**	0.85**	0.84**	1.00	0.93**	0.88**	0.85**
Day 11	-	1.00	0.97**	0.96**	-	1.00	0.96**	0.96**
Day 21	-	-	1.00	0.99**	-	-	1.00	0.99**
Day 30	-	-	-	1.00	-	-	-	1.00

** = highly significant at ≤1% level of significance

The ten markers thus identified were used to generate the genetic map of chromosome 3B at the LOD>2.0. The total length of the genetic map of chromosome 3B was 55.1 cM. Since four markers namely, *Xgwm181, Xgwm547, Xgwm247* and *Xgwm340* were cosegregating, the average intermarker distance was found to be 7.87 cM (Figure 58). The biggest gap of 22.9 cM was found between markers *Xbarc84* and *Xwmc291* that was same as the region where seed dormancy QTL was identified (Figure 58).

The genotypic data used to generate the linkage map was associated with the phenotypic data to identify the putative QTL for seed dormancy mapping on chromosome 3B. As a result only one QTL was identified spanning between the marker interval *Xbarc84-Xwmc291* (Figure 58), on the long arm of chromosome 3B of *T. diccoccoides*, for all the four field locations, for all the temperatures tested, explaining 39 to 54%, 46.78 to 58.49%, 35.71 to 52.53% and 49.57 to 56.81% of phenotypic variation for the dormancy trait recorded at Prosper 2006, Langdon 2006, Prosper 2007 and Langdon 2007 locations, respectively (Table 29). The QTL was consistently present for all the days 1, 11, 21 and 30. The distance between the marker interval *Xbarc84-Xwmc291* was 22.9cM (Figure 58). No QTL was identified for the Fall greenhouse season and Spring greenhouse material tested for dormancy at 27C. However, for the Spring greenhouse material tested for dormancy at 20C, QTL was detected on day 11, explaining 23.6% of variation for the dormancy trait (Table 29).

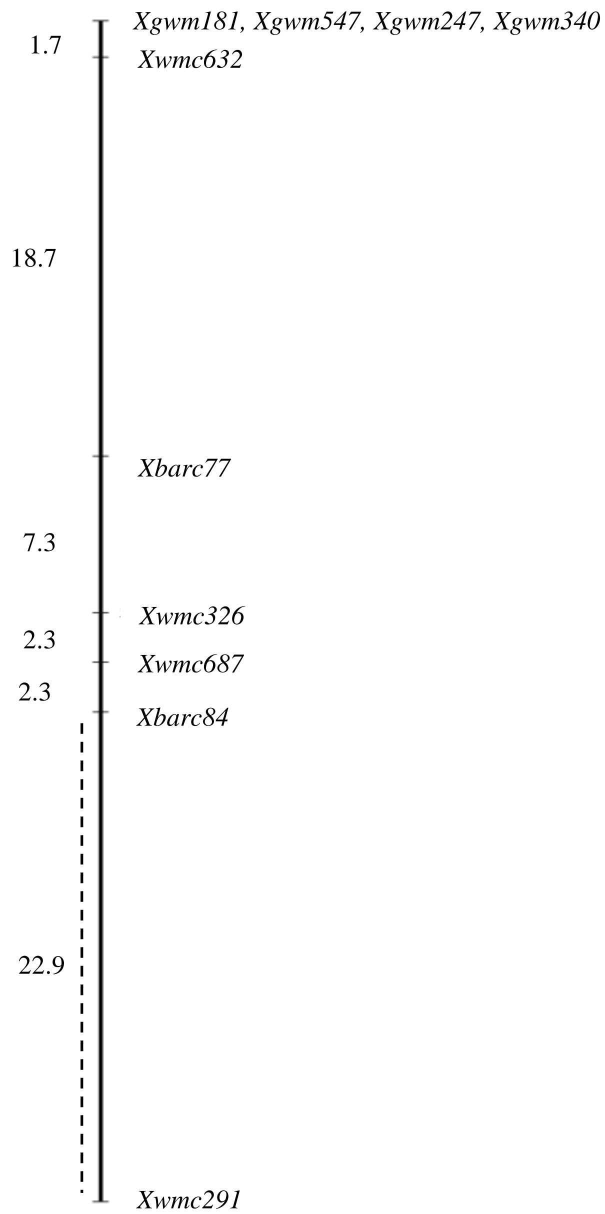


Figure 58. The linkage map of chromosome 3B constructed for the LDN (Dic-3B) RICLs. Dotted line indicates the position of the QTL for seed dormancy.

Table 29. The r^2 (%) for QTL detected between marker interval *Xbarc84-Xwmc291* for 91 LDN (Dic-3B) RICLs

	P06	L06	P07	L07	Spring (20C)
Day 1	41.06	46.78	52.53	54.57	-
Day 11	54.47	58.49	43.40	56.81	23.60
Day 21	46.90	53.61	37.18	53.61	-
Day 30	39.08	53.00	35.71	49.57	-

For the combined location analysis the QTL with main effect was identified for days 1, 11, 21 and 30, explaining 48.31, 53.07, 47.72 and 44.22 % of phenotypic variation for the dormancy trait (Table 30 and Figure 59, 60, 61 and 62). The QTLxE interaction was also observed for all the four days, thereby suggesting that the QTL might not respond evenly at different locations (Table 30). Since no QTL were observed for plant height and heading date, further analysis was not done for these traits.

Table 30. The r^2 (%) for QTL detected between marker interval *Xbarc84-Xwmc291* for 91 LDN (Dic-3B) RILs for combined location analysis

	Combined location	QTLxE
Day 1	48.31	2.82
Day 11	53.07	2.84
Day 21	47.72	1.23
Day 30	44.22	1.55

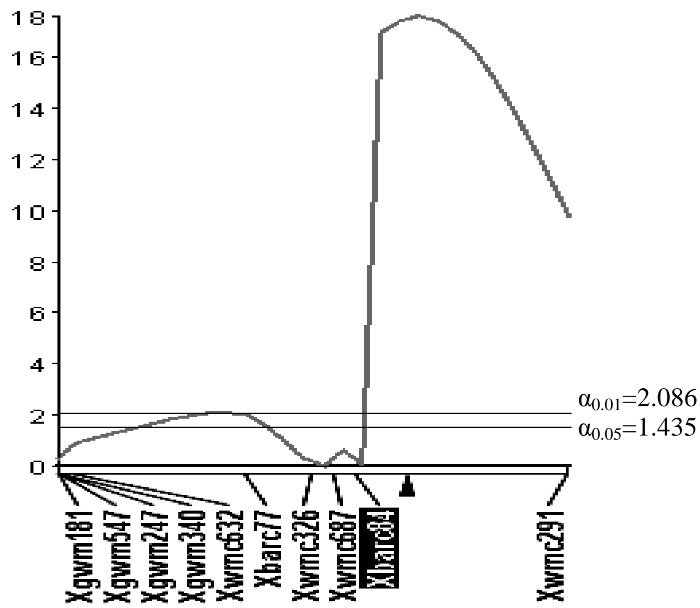


Figure 59. The QTL peaks for seed dormancy at Day 1 for LDN (Dic-3B) RICL population for combined field locations. Peaks above the LOD score at 0.01 and 0.05 level of significance calculated by 1000 permutations, are putative QTL.

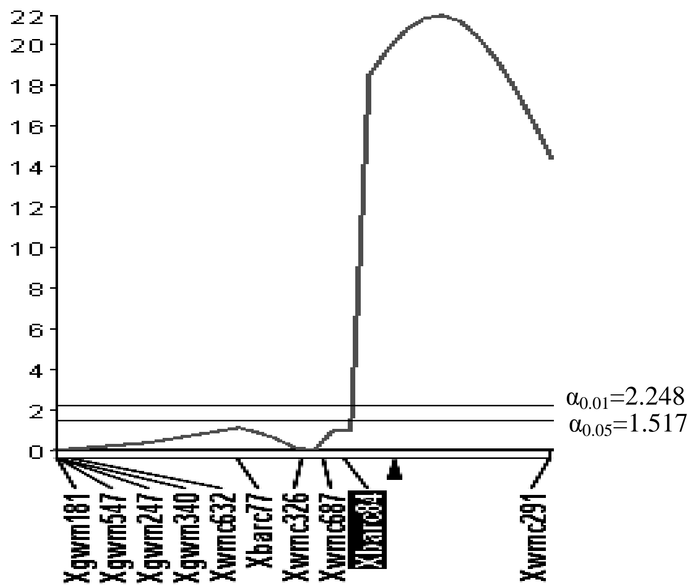


Figure 60. The QTL peaks for seed dormancy at Day 11 for LDN (Dic-3B) RICL population for combined field locations. Peaks above the LOD score at 0.01 and 0.05 level of significance calculated by 1000 permutations, are putative QTL.

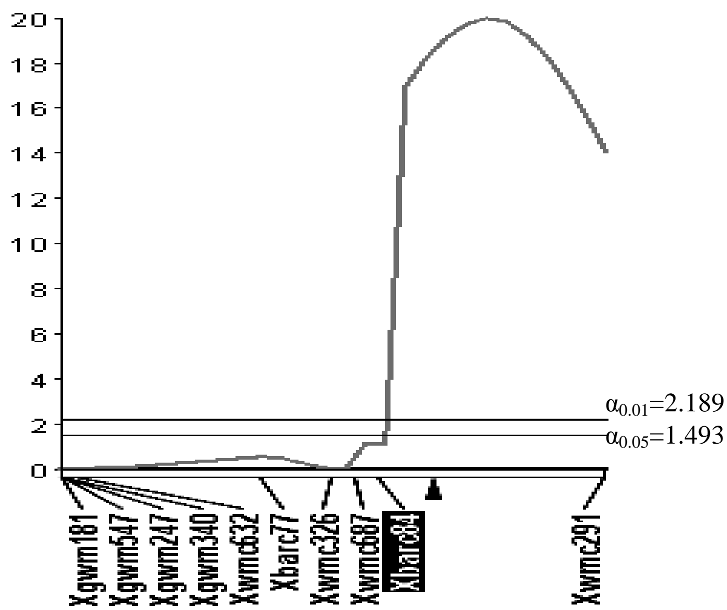


Figure 61. The QTL peaks for seed dormancy at Day 21 for LDN (Dic-3B) RICL population for combined field locations. Peaks above the LOD score at 0.01 and 0.05 level of significance calculated by 1000 permutations, are putative QTL.

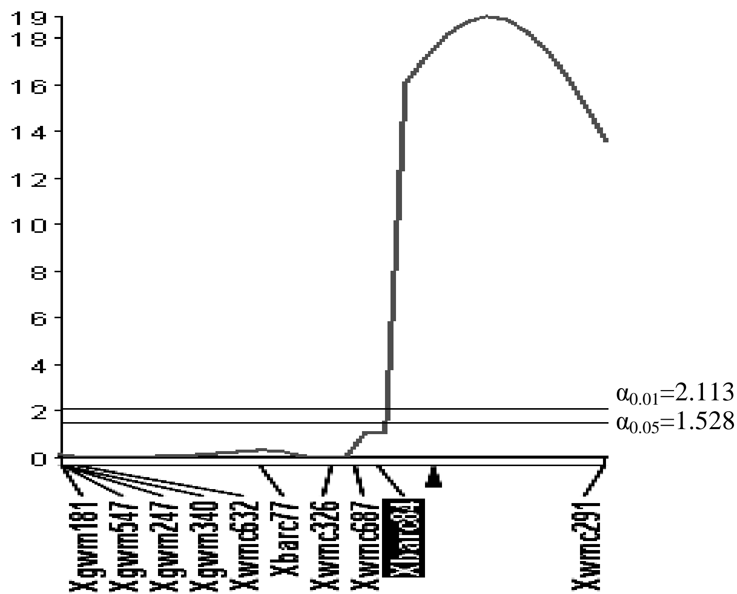


Figure 62. The QTL peaks for seed dormancy at Day 30 for LDN (Dic-3B) RICL population for combined field locations. Peaks above the LOD score at 0.01 and 0.05 level of significance calculated by 1000 permutations, are putative QTL.

DISCUSSION

Seed dormancy pattern on chromosome 3A

Seed dormancy was measured in population derived from the cross between LDN and LDN (Dic-3A) substitution lines by germinating hand threshed seeds for 30 days at 27^0C, which showed maximum variation between the parents. The germination experiments were performed for Spring and Autumn greenhouse seasons for year 2005 and for Prosper and Langdon field locations for year 2006. Seed germination was recorded daily for almost 30 days since nearly 100% germination was not obtained until day 30. Most researchers presented day 7 or day 15 data, which is when most of their seed material achieved 100% germination. Only germination data were presented at 10 days interval ie. day 1, 11, 21 and 30. Later data presented here was analyzed as cumulative germination percentage.

The frequency distribution curves for seed dormancy were obtained at 1, 11, 21 and 30 days of seed germination for both field and greenhouse seasons, in order to study the pattern of dormancy in the population. The cumulative seed germination data suggested that the population was found to be more dormant at day 1 and it became non dormant by day 30 for the seed material obtained from the two field locations. However, transgressive segregants were still observed for the dormant parent that could be attributed to the polygenic nature of the trait or overdominance. Thus, suggesting that the population was more like the dormant parent. The alleles for dormancy were contributed by the wild type parent *T. diccoccoides*, that is presumed to possess major genes for seed dormancy (Watanabe and Ikebata, 2000), that might have been lost in some present day cultivars as a result of domestication. By day 30 the dormancy was broken thereby suggesting that this dormancy was enough to overcome preharvest sprouting (PHS) and have better stand establishment when sown in the field. The greenhouse material was however, more nondormant over the time period examined. The QTL analysis also revealed presence of different QTL for field and greenhouse season that could have contributed to non dormant type of population for the greenhouse material (Table 12 and 13). This could be due to different temperature conditions during the seed setting stage that activated/deactivated different genes for seed dormancy in the greenhouse season. Reddy et al. (1985) tested for seed dormancy by growing seed material at different temperature conditions at the grain filling stage. As a result they observed that as they increased the temperature at the grain filling stage, the population became more non dormant.

The test for homogeneity of variance for day 1, 11, 21 and 30 revealed Prosper and Langdon field locations to be homogenous for these days, thereby allowing to combine the two field locations. The combined analysis for seed dormancy at field locations did not show any G X E effect. Absence of G X E effect means that with the change in location or environment the genotypes response to dormancy trait will not change. Therefore, the genes controlling dormancy trait in this population are strong and relatively consistent over different locations or field conditions.

QTL for seed dormancy on chromosome 3A

Seed dormancy is a complex trait controlled by many genes and influenced by environmental factors. Many QTL for dormancy have been identified on all 21 chromosomes of wheat (Paterson and Sorrells 1990; Mares 1996; Flintham et al. 2002, Anderson et al., 1993; Mares and Mrva, 2001; Kato et al., 2001; Roy et al., 1999; Varshney et al., 2001; Groos et al., 2002). Imitiaz et al. (2008) identified QTL for dormancy to be present on chromosome 3DL and 4AL of durum wheat. Mares et al. (2002) also identified two QTL for seed dormancy to be located on chromosome 2AL, 2DL, and 4AL.

Watanabe and Ikebata (2000) suggested that the group 3 chromosome posses the major genes for seed dormancy that are associated with the red grain color trait. These results were in accordance with the present research, since we identified putative QTL for seed dormancy located on chromosome 3A to be linked with the red seed coat color trait using both genotypic and phenotypic analysis. On the contrary the QTL for dormancy identified by Miura et al. (1996) located on chromosomes 3A, 3B and 3D of wheat using phenotypic analysis were not linked to red seed coat color. Osa et al. (2003) and Mori et al. (2005) also identified two QTL for seed dormancy on short and long arms of chromosome 3A in wheat that did not show linkage with red seed color trait. However, Groos et al. (2002) identified QTL for preharvest sprouting to be located on long arm of chromosome 3A and 3B of hexaploid wheat. These QTL were collocated with the QTL for grain color. The confidence interval of QTL detected for PHS and red grain color overlapped. Although, the effect of the QTL identified on chromosome 3A was underestimated due to the presence of gap in this region of the map.

Using single locus analysis (SLA) present research identified 6 QTL for seed dormancy for the field trials (Table 12) and 6 QTL for the greenhouse seasons (Table 13). Five QTL were identified for the greenhouse that were different from the ones identified in the field trials. This could be due to different

environment conditions in the field and the greenhouse that can activate/deactivate different genes at different stages of plant growth. Since the number of QTL identified were large, QTL analysis was performed for the combined field locations to identify QTL that were present over multiple environments and to detect if there are any QTL x E effect observed. Five QTL were identified for the combined location analysis (Table 14). The QTL between marker interval *Xcfa2193*-Xcfd2a was consistently present over multiple locations on day 1, 11, 21 and 30 (Table 12 and 14). The effect of this QTL was maximum on day 11 and was minimum on day 30 (Table 14). Thus, suggesting that this major QTL will provide strong dormancy under different environmental conditions and will reduce damage caused by PHS. However reducing effect of this QTL by day 30 signifies that it will also provide good stand establishment when seeds are sown in the field. This QTL identified in this research can open future prospects to develop cultivars with PHS resistance by incorporation in susceptible cultivars. However, more work needs to be done in order to narrow down the location of dormancy gene between the marker interval *Xcfa2193-Xcfd2a*. This QTL was also found to be associated with the red seed coat color identified by Nalam et al. (2006). Red seed coat color is not desired in the cultivars due to the lower consumer demand for the colored wheat for pasta production. However, while screening for red seed coat color trait, we observed that it was very hard to distinguish between white colored Langdon and red colored *T. dicoccoides* substitution lines. The red color did not develop well in the lines grown in North Dakota. However, Nalam et al. 2006 observed that the red seed color developed very well under Oregon conditions and were able to distinguish between the white and red seed coat color trait. This difference in the development of the red seed coat color could be attributed to different environmental and growth conditions between the two states. Thereby suggesting that the genes for seed dormancy identified in this QTL region can be incorporated in the modern day durum cultivars grown in North Dakota lacking seed dormancy without worrying about the fact that it is associated with red seed coat color trait.

The QTL identified between the marker interval *Xcfa2037-Xbcd115* was present only until day 11, thereafter it was not significant (Figure 20, 21, 22 and 23). Thus suggesting that this QTL will provide seed dormancy for only 11 days, thereafter seeds will germinate. For this reason this QTL is not a strong QTL and will provide resistance to PHS for a short time period. Two QTL spanning between marker interval *Xbarc45-Xgwm2* and *Xcfa2134-Xcfa2037* were only observed on day 21 and day 30 (Figure 22

and 23), however they were absent on day 1 and day 11 (Figure 20 and 21). Thereby, suggesting that these two QTL cannot prevent damage from PHS incase the conditions are favorable for PHS immediately after plant have reached physiological maturity. At day 21 and 30 the main effects of these QTL were found to be small, that might not be enough to provide resistance to PHS (Table 14). Fifth QTL identified between marker loci *Xbarc12* and *Xgwm369* was identified on day 11, 21 and 30 (Figure 21, 22 and 23). This QTL showed maximum effect on day 21 while the main effect decreased by day 30 (Table 14). This could be another potential QTL that could provide seed dormancy, thereby preventing losses caused due to PHS. Since it has decreasing main effect by day 30, it will provide better stand establishment for the seeds when sown in the field. Although this QTL is present on day 11 the small main effect observed on this day might not be enough to prevent losses caused by PHS.

Seed Dormancy QTL x E interaction on chromosome 3A

Dormancy is a quantitative trait that is influenced by the environment. QTL X E effect was observed for QTL ranging between marker intervals *Xcfa2193 - Xcfd2a* and *Xcfa2037 - Xbcd115* on day 1. Another QTL ranging between marker interval *Xbarc12 - Xgwm369* also showed QTLxE effect on day 21. This suggests that these three QTL will not behave the same over different locations for day 1 for the former two QTL and for day 21 for the later QTL. The multilocus estimates obtained on day 1 was significantly higher than the summation of the individual main effects of the QTL identified on that day, suggesting that QTL x E effect could have contributed in extrapolating the multilocus estimates for day 1. However, the multilocus estimates were lower than the summation of the individual main effects of the QTL for day 21 and 30. This could be attributed to the masking effect of these regions decreasing their total effect. In this study three types of QTL were identified based on time of expression and main effect. The early effect QTL was the one that showed main effect on day 1 and day 11 eg. *Xcfa2037 - Xbcd115*. This QTL will provide resistance to PHS immediately after harvest, however it will loose the resistance to germination later on. The late effect QTL like *Xbarc12 - Xgwm369*, *Xbarc45 - Xgwm2* and *Xcfa2134 - Xcfa2037* showed main effect after few weeks. These QTL will not provide resistance to PHS immediately after harvest rather at later stages. Third type of QTL detected in this study influenced the trait throughout the experiment eg. *Xcfa2193 – Xcfd2a*. This QTL will continuously provide resistance to PHS for a period of 30 days and represent the best chance in breeding effort.

The QTL identified in the present research explained maximum of 43.7% of variation for the trait that is greater than the previously reported QTLs for seed dormancy. In addition the genomic location of the QTL identified in this research is different from the ones identified in the previous research. This could be attributed to different sources of seed dormancy in different studies, varying methodologies of estimating seed dormancy and use of different marker densities. This study reports the potential of using linked markers to select for these regions. However, more work is required in order to saturate this region with more markers to further refine these QTL for use in marker assisted selection.

The QTL identified between marker interval *Xcfa2193* and *Xcfd2a* was consistently present over multiple field locations and was found to be linked to *Xcfd2a*. Thereby, suggesting that this marker could be further used in marker assisted selection (MAS) for the dormancy trait. MAS will allow selection of genotypes with dormancy in a segregating population based on the linked DNA markers at any plant growth stage. Although the QTL was consistently present over different environments, further study needs to be done to confirm if the marker is tightly linked to the gene of interest. This can be achieved if the marker can be used to identify the genotypes with strong dormancy from different cross combinations. Since the distance between the two markers is 4.4cM, more markers are required in order to saturate this region and to identify the co-segregating marker for the gene of interest. Once the marker that is tightly linked to the dormancy trait in this QTL region is identified it can be used to identify the dormant genotypes in different populations and different environments. This marker could be applied by the breeders in MAS to improve the quantitative traits of the present day cultivars. Mori et al. have used three allelic combination mapping on chromosome 3AS, 4AS and 4BS respectively and providing high level of dormancy.

Seed dormancy pattern on chromosome 3B

The pattern of inheritance for seed dormancy trait was studied in the population derived from the cross between LDN and LDN (Dic-3B) by germinating seeds at 27C for 30 days. The seed material to be analyzed was obtained from Fall 2005 and Spring 2006 greenhouse seasons and from Langdon and Prosper field locations for years 2006 and 2007. The number of seeds germinated was recorded daily for the period of 30 days. The cumulative germination percentage data has been presented at 10 days interval of 1, 11, 21 and 30 days of germination.

The frequency distribution curve was obtained for the dormancy trait for the seed material obtained from the field and greenhouse seasons. The population was more non-dormant for all the field and greenhouse seasons except for the fall greenhouse season and Langdon 2007 location that was more like dormant parent (Table 16, 18 and 21). This difference in the expression of the trait can be explained by the variation in the environmental conditions like temperature and moisture at different seed setting stages of the plant growth that result in changing the seeds from dormant to non dormant state. Similar results were presented by Reddy et al. (1985) and Lunn et al. (2002) when they observed that higher temperatures at seed development stage and lower temperatures at seed germination stage contributed towards producing non dormant seeds. In this research higher germination temperatures of 27^0C were used to measure seed dormancy. This high temperature could have masked the dormancy trait in the population as suggested by Nyachiro et al. (2002). The variation observed for Langdon 2007 location showing dormant population at higher germination temperature could be due to low field temperature at seed development stage as suggested by Reddy et al. (1985). The presence of GxE effect on day 21 and 30 for the dormancy trait also showed that the trait is influenced by environmental conditions in the population (Table F1).

Many transgressive segregants were observed for the dormant parent at Prosper 2006, Langdon 2006, Prosper 2007 and Spring season (Figure 25 to 40 and 44 to 50). For Fall 2005 season transgressive segregants were not observed until day 30 (Figure 43). Thus, suggesting the presence of either large number of dormancy genes or a major dormancy gene leading to overdominance. For Langdon 2007 location, although the population was more like the dormant parent, transgressive segregants were observed for both dormant and non dormant parents (Figure 37, 38, 39 and 40). Presence of transgressive segregants suggested that there are large number of genes for this trait segregating in the population. The genes for non dormancy were expressed possibly as a result of variation in environmental conditions. The presence of significant genotypic mean squares for all the field locations and greenhouse seasons suggested that genotypes in the population were different from each other for the dormancy trait (Appendix D Table D1, D2, D3, D4, D6 and D7). The large amount of variation present in the population provides wide genetic base for the efficient characterization of the seed

dormancy gene that could further provide resistance to PHS damage. The wide genetic base was contributed by the wild progenitor *T. dicoccoides* that possesses genes for dormancy.

QTL for seed dormancy and QTL x Environment interaction on chromosome 3B

Dormancy is a quantitative trait influenced by many genes and environmental conditions. In addition to that it has been linked to red seed coat color trait by many researchers. Watanabe and Ikebata (2000) suggested that chromosome 3B possesses strong genes for seed dormancy and is linked with red grain color trait. Kulwal et al. (2004) identified QTL for dormancy to be located on chromosome 2BL, 2DS, 3BL and 3DL. They also observed that the QTL located on chromosome 3BL was in close proximity to red color gene. Similar results were observed by Groos et al. (2002) when they identified QTL for PHS resistance on chromosome 3B between marker interval *Xgwm403 – Xgwm131* and close to the loci where R gene was previously mapped. The QTL identified on chromosome 3B in the present research was mapped between marker interval *Xwmc291 – Xbarc84*, that is distal to the QTL identified by Groos et al. (2002) between marker interval *Xgwm403 – Xgwm131*. The distance between *Xwmc291* and *Xgwm131* is about 12cM according to the wheat consensus SSR map published by Somers et al. (2004). Since the red grain color trait was not scored in the present study it is hard to deduce if the QTL identified between marker interval *Xwmc291- Xbarc84* was associated with this trait. Miura et al. (1996) study showed allele for seed dormancy located on chromosome 3B of wheat using phenotypic analysis but was not linked to alleles for red seed coat color trait. The QTL identified in the present research between the marker interval *Xbarc84 - Xwmc291* was identified in all the field locations (Figure 59, 60, 61 and 62).

Thus suggesting, it is a strong QTL that is consistently expressed over different environmental conditions. This QTL has the potential of providing resistance from PHS and thereby preventing the economic losses. The small number of QTL identified in this research was possibly due to inadequate genome coverage.

The QTL analysis performed over multiple locations revealed QTL X E effect, thereby suggesting that the environment will have some influence on the expression of this trait. The main effect of the QTL was found to be maximum at day 11 (Table 29 and 30). Thus, suggesting that this putative QTL will prevent damage from PHS throughout after seed setting to seed maturation and harvesting. Since its main effect is shown to be decreasing, it will provide good stand establishment when sown in field.

Future studies could use this research to observe if the QTL identified between marker interval *Xwmc291- Xbarc84* is linked to or is in close proximity of gene for red grain color and further narrow the location of the QTL for MAS.

Although this QTL showed QTL X Environment effect, it was still identified in multiple environments and over different years, thereby suggesting that this allele could be used in MAS. However, the distance between the two markers is large. More markers are required to saturate the identified QTL region in order to be used in MAS effectively. The current research would also provide the basis for further narrowing down this QTL region by high saturation mapping and identifying an allele closely linked to gene that could be used for improving the quantitative trait in the present day cultivars using MAS.

CONCLUSION

Pre harvest sprouting is one of the major problems in wheat that causes quality and yield losses, further leading to economic losses in the global market. In order to prevent losses caused by PHS, there is a need to develop cultivars with seed dormancy such that the seed does not germinate before harvest in the field itself. However, seed dormancy should not be too strong that it cannot provide stand establishment when sown in the field.

In the present research we identified QTL for dormancy on chromosome 3A and 3B of wheat by screening two populations derived by crossing LDN x LDN Dic-3A and LDN x LDN Dic-3B. The genes for seed dormancy in these two populations were contributed by the wild parent *T. dicoccoides*. The wild progenitor provides wide genetic base for seed dormancy trait, thereby leading to characterization of the dormancy gene.

Both the populations showed many transgessive segregants in the direction of the dormant parent for all the field and greenhouse seasons. However, few transgressive segregants also were observed in the direction of the non dormant parent. Thereby, suggesting that the population comprised of both dormancy as well as non dormancy genes. This could be attributed to different environmental conditions like high temperature during seed development stage that lead to non dormant population in certain cases like Prosper 2006, Langdon 2006, Prosper 2007 and Spring season for Population II. Also the combined analysis for both the populations for both genotypic and phenotypic analysis showed environmental effect to play role in the expression of the trait.

Five QTL for seed dormancy were identified on chromosome 3A for the QTL analysis performed over combined field locations. A pattern was observed for these QTL with regard to the occurrence of resistance to PHS damage. One QTL ranging between the marker interval Xcfa2037 and Xbcd115 showed resistance to PHS damage for only 11 days of germination period. Thereby, suggesting that this QTL will provide resistance immediately after spikes have reached physiological maturity. Three QTL ranging between marker intervals Xbarc12-Xgwm369, Xbarc45-Xgwm2 and Xcfa2134-Xcfa2037 showed resistance to PHS damage at 21 and 30 days of seed germination period. Thereby, suggesting that these three QTL will provide resistance to PHS damage after 21 days of physiological maturity. Only one QTL ranging between marker interval Xcfa2193 and Xcfd2a was consistently present for the 30 day period of

seed germination. This QTL being the strongest one should provide resistance from PHS throughout the growth season till harvest. This QTL was also found to be linked with the red grain color trait. However, if the association between the two traits is tight linkage or pleiotrophy is still unknown. Only one QTL identified showed early expression and three QTL showed late expression.

Only one major QTL was identified on the long arm of chromosome 3B between the marker interval *Xbarc84* and *Xwmc291*. This QTL was constantly present for all the field and spring greenhouse season for the seed germination period of 30 days. The QTL x E effect was also observed for this QTL, however it was very small. The effect of this QTL was found to be maximum on day 11 and reduced thereafter suggesting that it will provide resistance to PHS constantly but will also provide good stand establishment when sown in the field. The marker interval showing this QTL was large due to low marker coverage.

Further work needs to be done in narrowing down the marker interval showing the major QTL for seed dormancy and to validate the results by introgression into cultivated varieties

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APPENDIX A. ANOVA TABLES FOR RICLS DERIVED FROM CROSSING LDN X LDN (DIC 3A) FOR BOTH GREENHOUSE AND FIELD TRAILS

Table A.1 Mean squares from the anova for seed dormancy for population derived from the cross between LDN x LDN(Dic 3A) at Prosper and Langdon location for day1

Source	DF	Prosper	Langdon
Replications	1	23.1200	74.4200
Blocks within Replications (Adj.)	18	28.3644	11.8200
Component B	18	28.3644	11.8200
Treatments (Unadj.)	99	174.11**	26.0790**
Intra Block Error	81	32.6212	7.2447
Randomized Complete Block Error	99	31.8473	8.0766
Total	199	102.58	17.3659
Efficiency Relative to RCBD		97.6274	104.15

**Significant at .01

Table A.2 Mean squares from the anova for seed dormancy for population derived from the cross between LDN x LDN(Dic 3A) at Prosper and Langdon location for day11

Source	DF	Prosper	Langdon
Replications	1	109.52	44.1800
Blocks within Replications (Adj.)	18	104.63	206.80
Component B	18	104.63	206.80
Treatments (Unadj.)	99	936.70**	716.34**
Intra Block Error	81	125.47	139.75
Randomized Complete Block Error	99	121.68	151.94
Total	199	527.08	432.18
Efficiency Relative to RCBD		96.9802	102.67

**Significant at .01

Table A.3 Mean squares from the anova for seed dormancy for population derived from the cross between LDN x LDN(Dic 3A) at Prosper and Langdon location for day21

Source	DF	Prosper	Langdon
Replications	1	154.88	1971.92
Blocks within Replications (Adj.)	18	147.55	191.19
Component B	18	147.55	191.19
Treatments (Unadj.)	99	679.58**	646.37**
Intra Block Error	81	163.13	178.21
Randomized Complete Block Error	99	160.29	180.57
Total	199	418.61	421.30
Efficiency Relative to RCBD		98.2635	100.09

**Significant at .01

Table A.4 Mean squares from the anova for seed dormancy for population derived from the cross between LDN x LDN(Dic 3A) at Prosper and Langdon location for day30

Source	DF	Prosper	Langdon
Replications	1	141.12	4436.82
Blocks within Replications (Adj.)	18	107.21	244.98
Component B	18	107.21	244.82
Treatments (Unadj.)	99	356.84**	559.05**
Intra Block Error	81	177.94	216.14
Randomized Complete Block Error	99	165.08	221.39
Total	199	260.36	410.55
Efficiency Relative to RCBD		92.7727	100.28

**Significant at .01

Table A.5 Mean squares from the anova for seed dormancy for spring 2005 greenhouse population derived from the cross between LDN x LDN(Dic 3A) at day1 and day11

Source	DF	Day 1	Day11
Rep	2	457.5967	33.794239
Trt	80	1310.0683**	84.691770 ns
Error	160	73.6634	47.86091

ns=not significant, **Significant at .01

Table A.6 Mean squares from the anova for seed dormancy for fall 2005 greenhouse population derived from the cross between LDN x LDN(Dic 3A) at 20C

Source	DF	Day1	Day11	Day21	Day30
Rep	2	255.228271	4107.69172**	875.8961	140.1136
Trt	84	30.836692 ns	1043.48032**	1457.7247**	1596.5373**
Error	163	29.326442	381.9588	405.8254	323.4996

ns=not significant, **Significant at .01

Table A.7 Mean squares from the anova for seed dormancy for fall 2005 greenhouse population derived from the cross between LDN x LDN(Dic 3A) at 27C

Source	DF	Day1	Day11	Day21	Day30
Rep	2	1.19016806	854.12639	5932.69174**	6316.00798**
Trt	85	0.90495897 ns	392.40105 ns	1058.57578**	1006.62894**
Error	168	0.9382123	211.08580	342.5791	311.0317

ns=not significant, **Significant at .01

Table A.8 Mean squares from the anova for days of flowering for population derived from the cross between LDN x LDN(Dic 3A) at Prosper and Langdon location

Source	DF	Prosper	Langdon
Replications	1	45.1250	106.58
Blocks within Replications (Adj.)	18	2.4139	4.2133
Component B	18	2.4139	4.2133
Treatments (Unadj.)	99	12.0676**	14.9968**
Intra Block Error	81	0.7151	0.9207
Randomized Complete Block Error	99	1.0240	1.5194
Total	199	6.7397	8.7522
Efficiency Relative to RCBD		126.95	144.49

**Significant at .01

Table A.9 Mean squares from the anova for plant height for population derived from the cross between LDN x LDN(Dic 3A) at Prosper and Langdon location

Source	DF	Prosper	Langdon
Replications	1	655.22	640.82
Blocks within Replications (Adj.)	18	59.2700	154.08
Component B	18	59.2700	154.08
Treatments (Unadj.)	99	278.43**	205.98**
Intra Block Error	81	16.9126	86.1706
Randomized Complete Block Error	99	24.6139	98.5170
Total	199	154.05	154.70
Efficiency Relative to RCBD		128.80	105.85

**Significant at .01

APPENDIX B. LEVENE'S TEST FOR HOMOGENEITY RICLS DERIVED FROM CROSSING LDN X LDN(DIC 3A) FOR BOTH GREENHOUSE AND FIELD TRAILS

Table B.1 Mean squares for Levene's Test for Homogeneity of variance for population derived from a cross between LDN x LDN (Dic 3A) for prosper and Langdon 2006 locations

Source	DF	Day 1	Day11	Day21	Day30	
location	1	718821**	891674	718.2		2233300
Error	398	29702.2	257500	282060	245106	

Table B.2 Mean squares for Levene's Test for Homogeneity of variance for population derived from a cross between LDN x LDN (Dic 3A) for spring and fall greenhouse season analyzed at 20C

Source	DF	Day 1	Day 11
location	1	24990365**	41131414**
Error	488	244194	532520

Table B.3 Mean squares for Levene's Test for Homogeneity of variance for population derived from a cross between LDN x LDN (Dic 3A) for spring and fall greenhouse season analyzed at 27C

Source	DF	Day 1	Day 11
location	1	28871877**	5641624**
Error	494	228356	335030

Table B.4 Mean squares for Levene's Test for Homogeneity of days to heading (DTH) and plant height (HT) variance population derived from a cross between LDN x LDN (Dic 3A) for prosper and Langdon 2006 locations

Source	DF	DTH	HT
location	1	401.0	41.9386
Error	398	193.1	93243.7

APPENDIX C. ANOVA TABLES FOR COMBINED ANALYSIS FOR RICLS DERIVED FROM CROSSING LDN X LDN(DIC 3A)

Table C.1 Mean squares from the combined ANOVA for seed dormancy for field location between population derived from cross between LDN x LDN (Dic 3A)

Source	DF	Day11	Day21	day30
rep(loc)	2	76.8500	1063.4000	2288.97000**
loc	1	1082.4100	36100.0000**	69221.61000**
trt	99	1418.5732**	1090.0752**	708.38495**
loc*trt	99	234.4706	235.8788	207.50899
Error	198	136.8096	170.4303	193.2326

Table C.2 Mean squares from the combined ANOVA for days to heading and plant height between population derived from cross between LDN x LDN (Dic 3A)

Source	DF	Days to heading	Plant height
rep(loc)	2	75.852500**	648.02000**
loc	1	726.302500**	2872.96000**
trt	99	24.696237**	409.33778**
loc*trt	99	2.368157	75.07111
Error	198	1.271692	61.56545

APPENDIX D. ANOVA TABLES FOR RICLS DERIVED FROM CROSSING LDN X LDN(DIC 3B) FOR BOTH GREENHOUSE AND FIELD TRAILS

Table D.1 Mean squares from the anova for seed dormancy for population derived from the cross between LDN x LDN(Dic 3B) at Prosper and Langdon location at day1

Source	DF	Prosper (2006)	Langdon (2006)	Prosper (2007)	Langdon (2007)
Replications	1	165.62	591.68	544.50	141.12
Blocks within Replications (Adj.)	18	98.5978	135.24	82.1444	90.3644
Component B	18	98.5978	135.24	82.1444	90.3644
Treatments (Unadj.)	99	225.34**	388.25**	368.82**	439.98**
Intra Block Error	81	54.3410	97.0133	70.4062	79.8064
Randomized Complete Block Error	99	62.3877	103.96	72.5404	81.7261
Total	199	143.97	247.84	222.31	260.25
Efficiency Relative to RCBD		106.15	101.93	100.42	100.28

Table D.2 Mean squares from the anova for seed dormancy for population derived from the cross between LDN x LDN(Dic 3B) at Prosper and Langdon location at day11

Source	DF	Prosper (2006)	Langdon (2006)	Prosper (2007)	Langdon (2007)
Replications	1	1.2800	36.9800	353.78	633.68
Blocks within Replications (Adj.)	18	92.3022	252.09	190.51	208.52
Component B	18	92.3022	252.09	190.51	208.52
Treatments (Unadj.)	99	1044.17**	1178.50**	663.90**	854.88**
Intra Block Error	81	129.99	123.70	136.63	150.88
Randomized Complete Block Error	99	123.14	147.04	146.43	161.36
Total	199	580.73	659.63	404.90	508.75
Efficiency Relative to RCBD		94.7285	108.80	101.93	101.83

Table D.3 Mean squares from the anova for seed dormancy for population derived from the cross between LDN x LDN(Dic 3B) at Prosper and Langdon location at day21

Source	DF	Prosper (2006)	Langdon (2006)	Prosper (2007)	Langdon (2007)
Replications	1	89.7800	11.5200	505.62	1579.22
Blocks within Replications (Adj.)	18	76.2689	218.81	186.35	372.60
Component B BB	18	76.2689	218.81	186.35	372.60
Treatments (Unadj.)	99	755.04**	854.59**	654.95**	782.10**
Intra Block Error	81	115.99	88.5669	178.94	189.14
Randomized Complete Block Error	99	108.77	112.25	180.29	222.49
Total	199	430.19	481.05	418.06	507.71
Efficiency Relative to RCBD		93.7733	114.36	100.03	107.97

Table D.4 Mean squares from the anova for seed dormancy for population derived from the cross between LDN x LDN(Dic 3B) at Prosper and Langdon location at day30

Source	DF	Prosper (2006)	Langdon (2006)	Prosper (2007)	Langdon (2007)
Replications	1	176.72	7.2200	752.72	2231.12
Blocks within Replications (Adj.)	18	75.6756	226.46	198.59	408.68
Component B	18	75.6756	226.46	198.59	408.68
Treatments (Unadj.)	99	500.67**	787.27**	648.27**	732.97**
Intra Block Error	81	90.6311	90.6472	194.87	212.75
Randomized Complete Block Error	99	87.9119	115.34	195.55	248.37
Total	199	293.70	449.07	423.57	499.42
Efficiency Relative to RCBD		96.9997	114.73	100.01	107.38

Table D.5 Mean squares from the anova for seed dormancy for fall 2005 greenhouse population derived from the cross between LDN x LDN(Dic 3B) at 20C

Source	DF	Day1	Day11	Day21	Day30
Rep	2	-	96.515709	189.38805	21.5585249
Trt	93	-	48.335200	111.03285	7.1424757
Error	165	-	37.75537	91.98318	8.500598

Table D.6 Mean squares from the anova for seed dormancy for spring 2006 greenhouse population derived from the cross between LDN x LDN(Dic 3B) at 20C

Source	DF	Day1	Day11	Day21	Day30
Rep	2	11.42**	50.4400	24.8940	122.9745
Trt	92	2.69**	1849.3331**	1480.6911**	1219.9546**
Error	180	6.875492	131.8396	94.2715	77.0188

Table D.7 Mean squares from the anova for seed dormancy for spring 2006 greenhouse population derived from the cross between LDN x LDN(Dic 3B) at 27C

Source	DF	Day1	Day11	Day21	Day30
Rep	2	0.17391304	12.9848	63.2877	91.5616
Trt	91	0.32827542	2121.4532**	2209.8858**	1685.1724**
Error	177	0.35961680	92.0115	88.7011	99.9221

Table D.8 Mean squares from the anova for days of flowering for population derived from the cross between LDN x LDN(Dic 3B) at Prosper and Langdon location

Source	DF	Prosper (2006)	Langdon (2006)	Langdon (2007)
Replications	1	1.6200	2.0000	0.9800
Blocks within Replications (Adj.)	18	2.2256	1.7056	4.0633
Component B	18	2.2256	1.7056	4.0633
Treatments (Unadj.)	99	13.0816**	17.7564**	18.9180**
Intra Block Error	81	1.1521	0.7444	1.7022
Randomized Complete Block Error	99	1.3473	0.9192	2.1315
Total	199	7.1863	9.3009	10.4768
Efficiency Relative to RCBD		107.51	112.00	113.25

Table D.9 Mean squares from the anova for plant height for population derived from the cross between LDN x LDN(Dic 3B) at Prosper and Langdon location

Source	DF	Prosper (2006)	Langdon (2006)	Prosper (2007)	Langdon (2007)
Replications	1	141.12	946.13	4.2050	456.02
Blocks within Replications (Adj.)	18	82.5478	150.87	164.80	74.5422
Component B	18	82.5478	150.87	164.80	74.5422
Treatments (Unadj.)	99	288.60**	155.44**	147.28**	159.99**
Intra Block Error	81	25.9879	48.1201	56.0482	36.8299
Randomized Complete Block Error	99	36.2715	66.8018	75.8212	43.6867
Total	199	162.33	115.32	111.01	103.62
Efficiency Relative to RCBD		124.11	123.53	120.79	108.63

APPENDIX E. LEVENE'S TEST FOR HOMOGENEITY RICLS DERIVED FROM CROSSING LDN X LDN(DIC 3B) FOR BOTH GREENHOUSE AND FIELD TRAILS

Table E.1 Mean squares for Levene's Test for Homogeneity of variance for population derived from the cross between LDN x LDN(Dic 3B) at Prosper and Langdon locations for year 2006 and 2007

Source	DF	Day 1	Day 11	Day21	Day 30
location	3	539430**	2322429**	354046	1522420
Error	79	73803.5	301919	307016	296009

Table E.2 Mean squares for Levene's Test for Homogeneity of variance for seed dormancy for population derived from the cross between LDN x LDN(Dic 3B) for fall and spring seasons tested at 20C

Source	DF	Day 1	Day 11	Day 21	Day 30
location	1	16831.5**	57562865**	26679913**	13045499**
Error	532	1083.3	252870	303809	297964

Table E.3 Mean squares for Levene's Test for Homogeneity of variance for seed dormancy for population derived from the cross between LDN x LDN(Dic 3B) for fall and spring seasons tested at 27C

Source	DF	Day 1	Day 11	Day 21	Day 30
location	1	15.8910	70490581**	62639094**	31262237**
Error	528	2.5983	253705	358355	306118

Table E.4 Mean squares for Levene's Test for Homogeneity of plant height (HT) and days to heading (DTH) variance for population derived from the cross between LDN x LDN(Dic 3B) at Prosper and Langdon locations for year 2006 and 2007

Source	DF	HT	DF	DTH
location	3	140274	2	550.5
Error	796	53287.9	597	95.3214

APPENDIX F. ANOVA TABLES FOR COMBINED ANALYSIS FOR RICLS DERIVED FROM CROSSING LDN X LDN(DIC 3B)

Table F.1 Mean squares from the combined ANOVA for seed dormancy between population derived from cross between LDN x LDN (Dic 3B) for field material

Source	DF	Day21	Day30
rep(loc)	4	546.5350	791.95
loc	3	57126.4317**	73839.95**
trt	99	2183.8430**	1836.35**
loc*trt	297	287.6101**	277.60**
Error	396	155.9491	161.7935

Table F.2 Mean squares from the combined ANOVA for seed dormancy between population derived from cross between LDN x LDN (Dic 3B) for greenhouse material at 27C

Source	DF	Day1
rep(loc)	4	0.08928351
loc	1	1.06161579
trt	90	0.17134849
loc*trt	89	0.16185450
Error	333	0.19111972

Table F.3 Mean squares from the combined ANOVA for days to flowering between population derived from cross between LDN x LDN (Dic 3B)

Source	DF	Mean Square
rep(loc)	3	1.533333
loc	2	1181.760000**
trt	99	43.872323**
loc*trt	198	2.941818**
Error	297	1.465993

Table F.4 Mean squares from the combined ANOVA for plant height between population derived from cross between LDN x LDN (Dic 3B)

Source	DF	Mean squares
rep(loc)	4	386.86750**
loc	3	20454.66333**
trt	99	534.43354**
loc*trt	297	72.28960
Error	396	55.6453