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Title: Natural Outcrossing in Spaced Planted F_2 Populations and Solid Seeded Advanced Generations of Wheat (*Triticum aestivum* L. em Thell)

Abstract approved: Signature redacted for privacy.

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This study was conducted to investigate the possible contamination of foreign pollen at different stages of a wheat breeding program. These included spaced planted F_2 segregating populations, replicated yield trials and seed multiplication. The experimental wheat materials consisted of four different F_2 populations, four cultivars and two experimental lines.

Two genetic marker lines, Blue Hyslop and Blue Norco were used to determine the amount of possible outcrossing. The pollen of these marker lines carry genetic factors for blue aleurone color, thus the extent of outcrossing (wherever outcrossing had occurred in the experimental populations) could be immediately observed and determined during the same crop season.

The mean percent outcrossing recorded was less than 1% in spaced planted F_2 populations, different replicated yield trials

and seed multiplication conditions. The highest percentage of outcrossing of 5.6% was observed for a single plant in a spaced planted F_2 population. Under yield trial conditions, the highest percentage of outcrossing of 0.6% was recorded for the cultivar Hill 81 whereas the lowest value of 0.1% was recorded for experimental line SPN/AU/YMH.

Under seed multiplication conditions, a sample taken at 0.5 m away from blue aleurone pollen source in low density planting had a mean outcrossing value of 0.5% for the cultivar Jackmar. Pollen load of marker lines relative to that of all entries in low seeding rate was high, consequently, the low seeding rate had higher levels of outcrossing than the high seeding rate. No significant effect on outcrossing was observed for Seeding rate x Entry interaction. However, effects of entry and sampling distance on outcrossing were significant. As sampling distance increased from the blue aleurone pollen source, the level of outcrossing decreased.

Low positive correlation of 0.12 and 0.14 existed between outcrossing and plant height and tillers per linear meter respectively. Correlation of 0.70 was found between natural outcrossing and wind velocity.

Results of this study suggest that outcrossing can occur in all stages of a breeding program. The results also indicate that an isolation distance of 2 m established for multiplication of registered and certified seed in the certification program of Oregon State University might not be adequate especially for such cultivars as Jackmar and Hill 81. Furthermore, the level of outcrossing detected was high enough to distort and perhaps alter the genetic identity and integrity of the breeding material. A similar problem will exist if care is not exercised when growing valuable wheat germplasm in various collection centers.

Natural Outcrossing in Spaced Planted F_2 Populations and Solid Seeded Advanced Generations of Wheat (*Triticum aestivum* L. em Thell)

by

Jerry K. K. Boinnet

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Dedicated to: My grandparents KipBoinnet, KopCherotich and KopCheptanui for the tireless services they rendered onto our family; my wife Pamela for her patience, love, encouragement and sincere understanding in the entire period of my graduate studies and my children Kipruto and Kipkoech.

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INTRODUCTION

Plant breeding is defined as genetic adjustment of plants to the service of man. Genetic adjustment of plants depends on the understanding of their mode of pollination. Hence the choice of an effective selection procedure and the maintenance of genetic purity of superior genotypes is based on the type of pollination of the cultivar being improved.

Improvement in wheat productivity is partly dependent upon the development of high yielding cultivars. Through hybridization of an array of parents with desirable attributes and subsequent selection of promising progenies, new cultivars are developed to fit into a unique combination of soil, climate and biological conditions prevailing in individual localities. Such cultivar development is brought about by adopting one or a combination of breeding procedures suitable for self pollinating species. Although wheat is a self pollinating species, several studies have shown that wheat does outcross. Furthermore, many investigators have pointed out that outcrossing in wheat varies from genotype to genotype and from environment to environment.

Since outcrossing does occur in wheat, a need to study the impact and magnitude of natural outcrossing on genetic improvement procedures involving segregating populations in a pedigree selection system and the subsequent maintenance of genetic purity of improved cultivars is important.

Thus this study was conducted to determine whether: 1) the level of outcrossing in wheat is high enough to cause adverse effects on the routine handling of segregating populations and advanced generations in a wheat breeding program, and 2) the established minimum isolation distance for the multiplication and production of certified wheat seed is adequate. The factors investigated included: 1) extent of natural outcrossing in spaced planted F_2 populations of selected winter x spring and winter x winter crosses, 2) outcrossing in selected advanced generations of winter wheat when solid seeded in standard yield trial situations at low and high seeding rates, 3) correlation between outcrossing and such variables as wind velocity, plant height, days to heading and days to anthesis, and 4) the decrease in level of outcrossing with increasing distance from blue aleurone pollen source.

LITERATURE REVIEW

Natural Outcrossing in Self Pollinating Cereals

Although wheat is considered to be a self pollinating species, much pollen is shed at anthesis. Scientists working with wheat have on several occasions observed vigorous F_1 hybrids resulting from natural outcrossing occurring in their experimental fields. The amount of natural outcrossing and subsequent appearance of F_1 hybrids, has been considered to be of such a low frequency as not to have a significant effect on routine handling of either breeding stocks or improved cultivars. However, recent studies on natural outcrossing suggests that the amount and frequency of natural outcrossing is higher than previously thought (Allan, 1980).

As early as 1910s, natural hybridization between wheat and rye had been reported (Leighty, 1915). The rates of natural hybridization between wheat and rye was reported as ranging from 18% to 20% (Meister, 1921 and Leighty and Sando, 1928). In recent years, spontaneous hybridization between *Triticum* species and species of *Secale* and *Aegilops* has been noted (Dorofeev, 1969). Moreover, wheats of different ploidy level in the Near East have been observed to hybridize among themselves naturally (Zohary, 1971).

In a study of a population of barley for 18 generations, Jain and Allard (1960) and Allard and Jain (1962) pointed out that heterozygotes resulting from outcrossing had greater fitness and were maintained in the population from generation to generation. They also noted that natural outcrossing was one way of maintaining genetic variability in predominantly self pollinating populations. Natural outcrossing rates of 3 to 5% in barley have also been reported by Giles et al. (1974). In addition, Brown et al. (1978), in a study of outcrossing in 26 populations of wild barley estimated natural outcrossing to range from 0 to 9.6% and concluded that natural outcrossing was significantly higher in populations growing in more mesic than xeric regions.

In an intensive study of natural hybridization in common wheat conducted over a 10 year period, Leighty and Taylor (1927) observed different rates of cross pollination. In the strain 'Fulcaster', they recorded an unusually high natural outcrossing rate of 34%. 'Nebraska No 28' and 'Kanred' also were observed to outcross at relatively high frequencies. They concluded that natural hybridization varied from season to season and from cultivar to cultivar. However, natural outcrossing of less than 1% has been reported in wheat (Garber and Quisenberry, 1923; Poehlman, 1979 and Eske, 1981) but slightly higher frequencies of 3-4% have also been noted (Heyne and Smith, 1967 and Allan, 1980).

Wild emmer has a high cross pollination potential and it has been reported to outcross with cultivated wheats in the Near East (De Vries, 1971 and Zohary, 1971). Wheats developed in Mexico tend towards outcrossing (De Vries, 1971). 'Yorkstar' is reported to have had a great tendency for natural outcrossing and, subsequently, an isolation distance of 27 m was imposed while Yorkstar was being multiplied as certified seed (Jensen, 1968). In spite of the potential and the tendency of wheat to outcross, Wilson (1968) observed that with exception of a few soft white wheats, the outcrossing potential of various wheat classes was more or less the same provided extremes in environment factors were not encountered.

As cited above, predominantly self pollinating cereals such as wheat and barley outcross. Wheat hybridizes naturally with rye, other Triticum species and among different classes of cultivated wheats. The rates of natural outcrossing in self pollinating cereals are variable. Natural outcrossing is considerably important in maintaining genetic variability in self pollinating cereals.

Causes of Natural Outcrossing in Self Pollinating Cereals

Outcrossing in predominantly self pollinating cereals often occurs because 1) either the anther fails to dehisce inside the floret or 2) the pollen is non viable. Frankel and Galun (1977) pointed out that environmental factors such as temperature extremes, light quality and quantity, moisture stress and soil nutrient status influenced pollen and ovule development, floral sex expression and seed set.

To assess the influence of temperature on pollen development a prechilling study of wheat was conducted in a greenhouse by Suneson (1937). He discovered that temperatures between -2.8°C and 2.2°C killed wheat pollen and concluded that consistent low temperatures in the field favor outcrossing. Temepratures of 9°C imposed on wheat plants for 7 to 8 days resulted in an increased spikelet sterility and hence a decrease in the number of grains per ear (Balla, 1980). Frankel and Galun (1977) noted that in a wheat cultivar kept under constant illumination and low temperatures, the anthers were converted to carpellar structures.

In addition to the effects of temperature, moisture stress at certain stages of floral development is detrimental to the pollen development and viability. In an experiment conducted in a greenhouse, Bingham (1966) observed that a drought of 3 to 4 days preceding pollen meiosis in cultivar 'Cappelle Desprez' resulted in the lower florets of every spikelet being male sterile but female fertile.

Light quality and photoperiod also influence the development of the pollen and the ovule. Batch and Morgan (1974) reported an incidence of male sterility in barley which was induced by photoperiod. They noted that exposure of barley to short daylength (10 hours) resulted in male sterility. Fesenko (1968), cited by Goss (1968), established that light deficiency during the meiotic period resulted in production of much less pollen. Light, therefore, has a strong influence on the development of pollen.

All the above environmental factors are crucial at stages of floral development preceding meiosis. To understand how these environmental factors affect pollen development and viability, detailed microscopic examination of the pollen mother cell was conducted by Heslop-Harrison (1966). His examination of the meiotic division of the pollen mother cell revealed massive cytoplasmic connections formed in the meiocytes in the preleptotene stages, which disappeared prior to meiosis II. He pointed out that these connections were important in the movement of cellular materials in meiotic prophase. Thus his conclusion was that these connections accounted for synchronization of mother cell nuclei in meiotic division and that the sensitivity of the anthers to the injury at meiotic stage was due to traumatic effects of either the environmental or mechanical damage on the whole linked cell mass of the meiocytes.

Additionally, pollen malformation and malfunction is due to genetic factors. Under favorable environmental conditions 'Norin 10' (c.1 12699) was observed by Vogel et al. (1956) to have had low yields because a high proportion of its florets were sterile. Frankel et al. (1981) using speltoid series of mutations of T. aestivium, demonstrated that genetic systems which involve a major locus Q regulated floral fertility. These genetic systems interacted with the environment and caused base sterility. A male sterile vulgare wheat also appeared spontaneously in one F_3 family from a cross of 'Kenya Farmer' and 'Javelin 48' made by Australian breeders (Suneson, 1962). The male sterility resulting from this cross was reported to have been either due to the effect of homozygous recessive alleles at one locus or complimentary recessive alleles at two loci.

In general, pollen development and viability are affected either by genetic or environmental factors, or a combination of both. The non-viable pollen greatly enhances the outcrossing potential of self pollinating cereals.

Pollen Production, Dispersal and Seed Set

For outcrossing to occur in predominantly self pollinating cereals, pollen from male fertile florets has to be released external to the floret and dispersed by wind to either a male sterile floret or an open floret whose anthers have not dehisced and whose stigma is receptive. Wind at high velocities agitates the anthers, causing more pollen to be released and carried to greater distances. Pollen dispersal and outcrossing are influenced by such factors as: distance between pollen source and male sterile florets, presence or absence of natural or artificial barriers, wind velocity, relative population sizes of two or more different cultivars, and cultivar differences in the time of flowering.

For the pollen to be released outside the flowers of fertile florets, the opening of the fertile florets and the elongation of the filaments have to be synchronized. Nishiyama (1970) identified two stages of flowering in *Triticum* species: the maturation of the sexual organs and the opening of palea and lemma. He noted that the maturation period was shorter in tetraploids and hexaploid wheats but longer in diploid wheats. At flowering time, lodicules become very turgid and push lemma and palea apart (Nishiyama, 1970 and De Vries, 1971). At the same time, the filaments elongate, forcing the anthers to leave the florets. As the anthers extend out of the floret dehiscing, pollen is released both inside and outside the floret. The duration in which the florets stay open is variable and is influenced by the genetic make up of the cultivar and such environmental factors as temperature and humidity. The average duration of floral opening has been observed to last for 8 to 30 minutes (Percival, 1921). Fertile florets stay open at their widest only for 5 to 15 minutes (De Vries, 1971). In general terms, fertile florets stay open for a short while whereas sterile florets stay open longer.

Successful cross pollination is enhanced by using cultivars which head at nearly the same time and have anthesis occurring for a fairly long duration. In Washington state, U.S.A., cultivars planted 75 days apart flowered within 5 days of one another (Allan, 1980). Flowering duration and patterns have been studied by many investigators. Selected cultivars of winter wheat were observed by Balla (1980) to flower for 7 to 10 days in the field and for 14 to 16 days in the greenhouse. He noted that when conditions were more favorable for plant development the flowering period was longer. In a study of 5 spring wheats, an average flowering duration of 8.6 to 10 days was observed (De Vries, 1973). In the midwestern states of U.S.A., flowering duration lasting for 7 to 12 days has been noted (Patterson, 1970). Flowering pattern has been reported to be diurnal with two bimodal peaks at hours 08.00 to 11.00 and 15.00 to 18.00 (Patterson, 1970 and De Vries, 1971).

Outcrossing is favored by excessive shedding of pollen. Pollen shedding capacity in wheat is highly correlated with filament length, anther size, pollen grains per anther and the extent of anther extrusion (Beri and Anand; De Vries, 1974; Joppa et al., 1968; and Yeung and Larter, 1972). Taller wheat plants tend to have longer filaments, more pollen grains per anther and shed greater quantities of pollen outside their florets (Beri and Anand, 1970). Anther extrusion in wheat is controlled by a few genes with additive effects and low heritability (Sage and Isturiz, 1974). Average number of pollen grains per anther in wheat varies from 581 to 3867 (De Vries, 1971). Relative to other cereals, wheat has been ranked low in pollen production (Patterson, 1970). However, different classes of wheat have varied pollen production capacities. Joppa et al. (1968) noted that spring bread wheat shed more pollen than spring durum wheats. Conversely, Svensson (1972) observed that winter wheats shed more pollen than spring wheats and hence winter wheats were better pollinators in hybrid seed production. Furthermore, pollen shedding capacity in wheat is remarkably influenced by environmental factors, especially temperature and humidity. De Vries (1972) reported that most pollen was shed at temperatures of between 16 and 20°C and relative humidity of between 70 and 75%.

Once pollen has been released outside a fertile floret, it has to be dispersed and carried by the wind to a male sterile receptive floret so as to ensure successful cross pollination. The density and aerodynamics of wheat pollen are among the most important factors influencing dispersal. The distance to which pollen is dispersed depends on its density and the speed of the prevailing winds. Whitehead (1968) pointed out that lighter pollen grains were dispersed to a greater distance as they settled less rapidly. He also observed that wind velocity increased with height and that the extent of pollen dispersal was proportional to the height of its source. Wheat pollen, relative to that of other cereals, is medium in size (Patterson, 1970), but its density has not been fully established by scientists. Jensen (1968) and De Vries (1972) reported that wheat pollen was heavy and settled rather rapidly. However, Arp (1967) and Curtis and Johnston (1969) noted that wheat pollen was heavier than air but was easily borne aloft by air currents and carried to greater distances by wind.

Nevertheless, pollen dispersal in many cereals follow a common trend (Jones and Newell, 1946 and Bateman, 1947). With the wind dispersing the pollen grains away from its source and with the force of gravity acting upon them, a rapid decrease in pollen load occurs. Bateman (1947) observed that at a distance of 18 m away from pollen source, the atmospheric pollen had dropped to 1%. Jones and Newell (1946) recorded a pollen concentration of 0.8% at 300 m away from a cereal field. However, they stressed that in open pollinated cereals, the low pollen concentration at 300 m would still endanger the genetic purity of the cultivar.

For successful cross pollination to occur, pollen has to 1) remain viable while being dispersed, and 2) settle on the receptive stigma of a male sterile floret. Curtis and Johnson (1969) reported that wheat pollen under laboratory conditions had a short life span of 5 minutes at a temperature of 35°C and relative humidity of 20%. In addition, Arp (1967) observed that wheat pollen viability was low when temperatures were high and when relative humidities were either low or high.

A leveling off in productivity has occurred in several crop species. In an effort to break through these apparent yield plateaus, breeders of self pollinating cereals have ventured into researching the possibilities of producing hybrid wheat. Consequently, a number of studies have been conducted to investigate the factors that hinder seed set on either cytoplasmic male sterile lines or chemically induced male sterile lines.

Eske (1981), investigating male sterility induced by gameticides and subsequent seed set on induced male sterile lines of wheat, noticed that seed set in treated unbagged spikes was significantly higher than in treated bagged spikes. There was no significant difference in seed set of untreated bagged spikes. He concluded that outcrossing was important in bringing about significant seed set in treated and unbagged spikes. In addition, Jensen (1968) pointed out that seed set on cytoplasmic male sterile lines of wheat drops rapidly after a distance of 1.5 m away from the pollen source. However, Miller and Lucken (1976) observed no difference in seed set on male sterile strips of 1.5 m to 3.1 m wide. This finding was supported by Bitzer and Patterson (1968) who obtained a seed set of 70% on cytoplasmic male sterile of even wider strips (1 to 5 m). On the other hand, Wilson and Ross (1968) noted a small difference in seed set in different ratios of male sterile to male fertile strips and concluded that pollen load was not the limiting factor in seed set on cytoplasmic male sterile lines of wheat. Furthermore, wheat pollen is reported to be similar in size to that of sorghum and is therefore effective in causing cross pollination over greater distances (Wilson, 1968). In his experiments he obtained a seed set of 10% on cytoplasmic male sterile lines of wheat at a distance of 30 m away from a pollen source. Moreover, Yeung and Larter (1972), reported a small percentage of seed set on hand emasculated triticale plants at a distance of 30 m, away from the pollinator.

Open or closed flowering type of cytoplasmic male sterile lines affect seed set. Keydel (1977) observed that average outcrossing over a 5 m distance in an open flowering type of cytoplasmic male sterile line of wheat was 0.20% whereas in closed flowering type, the average outcrossing was 19.33%. He concluded that factors such as varietal earliness and height were also important in influencing cross pollination in self pollinating cereals and that under their environmental conditions an isolation distance of 5 m was inadequate for varietal maintenance in wheat. In Oregon State, the certification agency imposes an isolation distance of 27 m while foundation seed is being multiplied and 2 m while registered and certified classes (of seed) are being increased (Oregon State University, Extension Service, 1981).

In summary, the literature review does indicate that: wheat cultivars do outcross at different rates, wheat cultivars head at nearly the same time at high latitudes, environmental and genetic factors influence the potentials of wheat to outcross, pollen production and shedding capacity of wheat are variable and pollen dispersal and subsequent seed set is effective over a distance of 30 m but decrease with increasing distance away from a pollinator. Hence, the questions which need to be answered are:

1) Does the level of natural outcrossing have any detrimental

effect on the routine handling of segregating populations and seed increases in a wheat breeding program?

2) Is the established minimum isolation distance for the production of certified wheat seed adequate?

MATERIALS AND METHODS

Three studies were conducted to investigate the extent of natural outcrossing in wheat. Study I was designed to determine the extent of natural outcrossing in spaced planted F_2 populations. The objective of Study II was to evaluate the rate of natural outcrossing in advanced generations when grown under standard yield trial conditions. Study III was conducted to investigate the extent of natural outcrossing in advanced generations of winter wheat when seeded at low and high seeding rates in seed multiplication conditions.

Four cultivars, two experimental lines and four F_2 crosses were used in these studies. The four cultivars of winter wheat were: 'Stephens', 'Yamhill', 'Jackmar' and 'Hill 81'. Whereas the two experimental lines were 'SPN/AU/YMH' and '55.1744/7C//SU/RDL'. Similarly, the four F_2 crosses used were: 'SDY/HER', 'MHB/VEE', 'VORO/ASP' and 'STP/YR//BUC'. Three of the F_2 populations resulted from winter x spring crosses and one from a winter x winter cross.

The selection of these experimental materials was based on the diversity of the pedigree and the phenotype. The intent was to have cultivars, experimental lines and F_2 populations which represent a wide array of genetic backgrounds. For instance, Stephens is an awned soft white winter wheat whereas 55.1744/7C//SU/RDL is an awned hard red experimental line with a pedigree representing American, Mexican and Korean genetic sources. On the other hand, SDY/HER resulted from a winter x spring cross with both parents being early maturing, yet, STP/YR//BUC is a winter x spring cross with

'Strampelli' (STP) and 'Yecora' (YR) being early maturing and 'Buckbuck' (BUC) being late maturing. Specific characteristics of each cultivar, and parents of the experimental lines of F_2 populations are provided in the Appendix Table 1.

A blue aleurone genetic marker from the cultivar 'Baart', was backcrossed into the cultivars 'Norco' and 'Hyslop'. The resulting genetic marker lines, Blue Hyslop and Blue Norco were subsequently used to determine possible outcrossing in the studies conducted. The blue aleurone color which was carried by the pollen of the two marker lines is controlled by a single dominant gene. This gene exerts a blue xenic effect on the aleurone layer of any seed fertilized by pollen of the genetic marker lines. Hence, the outcrossed seed could immediately be determined on the basis of color at harvest time in the spikes of the plants sampled.

Location

The three studies were conducted at Hyslop Experiment Farm in 1981/82. Hyslop Experimental Farm is located in the Willamette Valley in western Oregon near Corvallis. Hyslop Farm has a mean annual precipitation (rainfall) of 994 mm and a mean annual temperature of 10.6°C. A total of 1383.5 mm was received in the form of rain and snow, during experimental period. Rainfall persisted throughout spring but decreased towards harvest time. Details of the weather conditions prevailing during the entire period of this research are summarized in Appendix Tables 2 and 3. The soil type is a Woodburn silt clay loam, with a pH of 5.5.

Methods

Prior to planting, a mixture of methyl bromide and chloropicrin at the ratio of 63:33 was applied at the rate of 420.40 kg/ha to control weeds and soil borne diseases such as root rot (*Pseudocercosporella herpotrichoides*) and take all (*Gaeumannomyces graminis* var. *tritici*). To control *Septoria tritici* and *Septoria nodorum*, the experimental field was sprayed three times, using a back pack sprayer, with Bravo 500 at the rate of 0.89 kg/ha (active ingredient). The spraying was done on April 20, May 4 and May 18. These sprays were carried out when the crop was at elongation and booting stages. Using a manually operated 'Seymour Universal' fertilizer spreader, urea at the rate of 50 kg N/ha was applied on April 8 and 30. Fertilizer was applied at tillering and booting stages. Prior to planting, urea at the rate of 33 kg N/ha was applied. No phosphorus was applied as soil analysis showed that the amounts of phosphorus present in the experimental field was adequate.

All materials were arranged as illustrated in Figure 1.

Study I

Four F_2 populations served as experimental materials for this study.

The four populations were fall planted in a randomized block

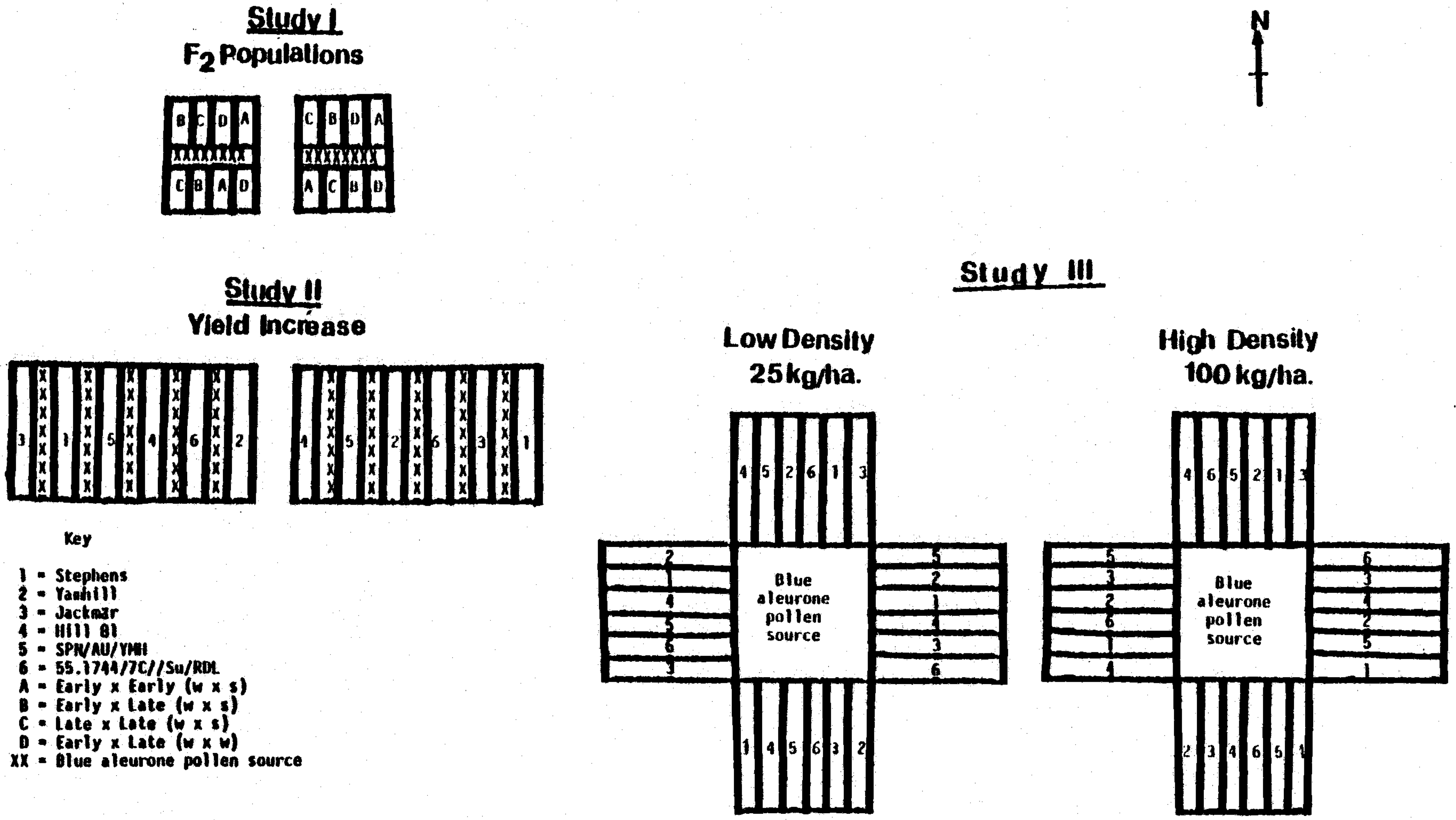

Figure 1. Experimental Layout

design with four replications. Each population was planted in four rows 30 cm apart. Twenty plants were spaced 10 cm apart within the rows. The total plot size was 1.2 m x 2 m. Genetic marker lines were planted between the replicates in 32 rows spaced 30 cm apart. Six plants spaced at 10 cm were planted in each row. The total area occupied by the genetic marker lines was 0.6 m x 9.6 m. In order to synchronize flowering periods of the marker lines and the F_2 populations, planting was carried out on October 24, November 4 and November 20. Depending on the relative earliness of the parents and genetic marker lines, late flowering populations were planted earlier and earliest flowering populations were planted later.

Measurements

Days to Heading

Days to heading was taken as the number of days from first of January to the date when 50% of the spikes in each plot had emerged from the flag leaf sheath.

Days to Anthesis

Days to anthesis was recorded as the number of days from first of January to the date when 50% of the spikes in each plot were shedding pollen (as measured by anther extrusion outside the floret).

Plant Height

Plant height was measured in cm from ground level to the tip of the tallest culm excluding the awns when present, two weeks after anthesis. The mean height in each plot was computed and recorded.

Tillers Per Plant

At harvest time, the number of spike-bearing tillers was counted for each plot. The mean number of tillers per plant for each plot was computed and recorded.

Natural Outcrossing Per Plant

After threshing the spikes of each plant in a single head thresher, and cleaning the seed, blue seeds were separated and counted. The ratio of blue seeds to the total number of seeds for each plant was multiplied by 100 and recorded as natural outcrossing per plant.

Mean Outcrossing Per Cross

After the spikes of the 80 plants from each plot had been threshed, cleaned and put into 'coin envelopes', seed samples with blue seeds were separated from those which had no blue seed and counted. The ratio of the seed samples with blue seeds to the total number of seed samples in each plot was multiplied by 100 and recorded as mean outcrossing per F_2 cross.

In addition, data on: days to heading, days to anthesis and plant height for the marker lines were taken as described above.

Study II

The extent of natural outcrossing in selected four winter cultivars and two experimental lines grown under standard yield trial situations was investigated.

The six entries were fall planted in two replicates in a randomized block design. The seeding rate for all entries was 100 kg/ha. Each entry was planted in four rows 20 cm apart. The total plot size was 0.8 m x 10 m. Also in between the entries the marker cultivars were seeded at the rate of 100 kg/ha and occupied a total plot size of 0.8 m x 10 m. The entries and the marker cultivars were planted on October 24, 1981.

Measurements

Days to heading, days to anthesis and plant height were measured as described in Study I. However, unlike Study I, all these measurements were taken on plot, rather than plant basis.

Fertile Tillers Per Linear Meter

Two samples of one linear meter were randomly taken within each of the two rows adjacent to the genetic marker lines. The number of fertile tillers in each sample was counted. The mean was computed and recorded.

Percent Natural Outcrossing

Two samples of one linear meter which were randomly harvested from each of the two rows adjacent to the marker cultivars were threshed, cleaned and the blue seeds and white/red seeds were separated. The ratio of blue aleurone seeds to the total number of seeds in each sample was multiplied by 100 and the mean was recorded as the value for outcrossing in each entry.

Similarly, data for days to heading, days to anthesis and plant height for the genetic marker lines were taken as described in Study I (but on plot rather than plant basis).

Study III

The extent of natural outcrossing in four winter wheat cultivars and two experimental lines solid seeded at low and high densities was evaluated using the same experimental material as in Study II.

The six entries were fall planted in four replicates in randomized block design. This study was divided into two parts, involving two seeding rates. The first planting density was 25 kg/ha. Each entry had two rows spaced 20 cm apart. Plot size was 0.4 m x 10 m. The second planting density was 100 kg/ha. Each entry had eight rows spaced 20 cm apart. Plot size was 1.6 m x 10 m. The marker lines in both planting densities was seeded at the rate of 100 kg/ha at the center of all the replicates (see experimental layout Figure 1). The total area occupied by marker lines in both parts of the study was 10 m x 10 m. To achieve proper 'nicking' of all entries and the marker lines at flowering time, the marker lines were planted on October 24 and November 13. Within each plot five sampling distances were considered as sub-plots. The intent of these different sampling distances was to investigate the effect of distance on natural outcrossing.

Measurements

Days to heading, days to anthesis and plant height were recorded following the procedure stated in Study I (but on plot rather than plant basis).

Tillers Per Linear Meter

In each planting density, two rows of 0.5 m in length (in the case of high density the two middle rows) were harvested within each entry and the number of tillers were counted and recorded as tillers per linear meter. The two rows were harvested at distance of 0.5 m, 1 m, 2 m, 4 m and 8 m away from the blue aleurone marker lines. Total area harvested at each specific distance was 0.4 m x 0.4 m.

Percent Natural Outcrossing

The harvested portions of each entry in each planting density, were threshed, cleaned and the blue seed (when present) was separated from the white/red seeds and counted. In each entry, ratio of blue seed to the total number of seeds in each distance was multiplied by 100 and recorded as percent natural outcrossing.

Following the procedure already described, data for the days to heading, days to anthesis and plant height for the marker lines were taken and recorded.

Wind direction and speed, solor radiation, temperature, humidity and rainfall were all obtained from Hyslop weather station.

Statistical Analysis

Data collected from Studies I and II were analyzed in a randomized complete block design. Unless otherwise stated, F value at 5% probability level was used to test whether differences existed between treatment means. The least significant difference (LSD) was used to determine if treatment means were significantly different.

Further, data from Study III was analyzed in a split-split plot design with seeding rate as the main plot, entries as sub-plot and sampling distances as sub-sub plot. The degree of association between natural outcrossing and the measured variables was assessed by the use of coefficients of correlation. Regression analysis was utilized to measure the change in the level of outcrossing associated with a change in sampling distance.

EXPERIMENTAL RESULTS

Results of this investigation will be presented for each of the three studies conducted. In Study I information regarding selected agronomic traits, mean percent natural outcrossing per plant and percentage of outcrossed plants in each F_2 population will be presented. Besides, data on the same agronomic traits and percent outcrossing per linear meter for each entry in Studies II and III will also be provided.

Study I

Agronomic Traits

In Table 1, observed mean values for both heading and anthesis dates in spaced planted F_2 populations indicated that significant differences existed between some populations. The earliest population was SDY/HER which headed and reached anthesis in 133 and 138.8 days respectively. In contrast, the population VORO/ASP was the latest, heading and reaching anthesis in 144.5 and 150 days respectively. The genetic marker Blue Hyslop headed in 144 days and reached anthesis in 148 days, whereas the other genetic marker Blue Norco headed in 152 days and reached anthesis in 156 days. Flowering synchronization between populations SDY/HER, STP/YR//BUC and MHB/VEE and genetic marker lines was poor.

Differences between population means for plant height were also

Table 1. Mean values for four agronomic traits involving four F_2 populations, two genetic marker lines and percent outcrossing when spaced planted at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

F_2 population and marker lines	Days to heading	Days to anthesis	Plant height (cm)	Tillers per plant	Percent Outcrossing per plant	Percentage of outcrossed plants per population
SDY/HER	133.0	138.8	81.4	18.4	0.13	28.3
STP/YR//BUC	139.0	144.5	95.0	15.5	0.45	63.0
MHB/VEE	141.5	146.5	97.0	17.0	0.32	60.3
VORO/ASP	144.5	150.0	104.0	16.0	0.44	52.3
*Blue Hyslop	144.0	148.0	98.5	--	--	--
*Blue Norco	152.0	156.0	144.0	--	--	--
LSD.05	3.1	2.3	6.7	N.S.	0.17	16.3

*Blue = genetic marker line

N.S. = differences between the means not statistically significant

significant (depending on the cross, see Table 1). The population SDY/HER had the lowest mean value for plant height of 81.4 cm whereas the population VORO/ASP had the highest value of 104 cm. Genetic marker lines Blue Hyslop and Blue Norco had mean plant height of 98.5 cm and 144 cm respectively.

Tillers per plant in the F_2 populations varied (Table 1). The population STP/YR//BUC had the lowest mean value of 15.5. Conversely, population SDY/HER had the highest value of 18.5. The differences in mean values of tillers per plant in F_2 populations were not significant. Although the mean values for tillers per plant in F_2 populations were relatively low, as many as 56 tillers were observed in a single plant involving SDY/HER population.

Percent Natural Outcrossing

Differences between population mean values for percent outcrossing per plant were significant (Table 1). An examination of the data reveals that the mean outcrossing per plant in all F_2 populations was less than 1%. The cross SDY/HER had the lowest value of 0.13% whereas the cross STP/YR//BUC had the highest value of 0.45%. Although the mean outcrossing values for all F_2 populations was less than 1%, a high value of 5.6% was observed for a single plant in the population VORO/ASP.

When considering outcrossing per population SDY/HER had the lowest percentage with 28.3% of the plants exhibiting the blue outcrossed seed. Conversely, in the population STP/YR//BUC, 63% of the plants were found to contain blue seed.

Additionally, the data in Table 1 showed that SDY/HER, which had the lowest values of mean outcrossing, also had the lowest ratio of outcrossed plants. Similarly, STP/YR//BUC, which had the highest value of mean outcrossing, had the highest ratio of outcrossed plants. However, the population MHB/VEE, which had a relatively low value of mean outcrossing of 0.32%, had a relatively high ratio of outcrossed plants of 60%. Furthermore, the population VORO/ASP, which had a comparatively high mean outcrossing value of 0.44%, had a moderate ratio of outcrossed plants of 52%.

Study II

Agronomic Traits

The means for heading and anthesis dates of winter wheat cultivars and experimental lines grown in standard yield conditions are presented in Table 2. Experimental line SPN/AU/YMH was the earliest heading and reaching anthesis in 142 and 145 days respectively. Experimental line 55.1744/7C//SU/RDL was the latest, heading and reaching anthesis in 150 and 155.5 days respectively. In addition Blue Hyslop headed and reached anthesis in 144 and 149 days respectively while Blue Norco headed in 152 days and reached anthesis in 156 days. As compared with the experimental lines and

Table 2. Mean values for four agronomic traits involving four winter cultivars, two experimental lines and two genetic marker lines and percent natural outcrossing when planted in standard yield trial conditions at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Entry and marker lines	Days to heading	Days to anthesis	Plant height (cm)	Tillers per linear meter	Outcrossing per linear meter (%)
Stephens	144.00	147.50	108.50	110.50	0.36
Yamhill	147.50	150.50	125.50	69.50	0.21
Jackmar	147.50	150.50	105.50	91.50	0.25
Hill 81	149.50	153.00	113.00	79.00	0.55
SPN/AU/YMH	142.00	145.00	106.00	82.50	0.10
55.1744/7C//SU/RDL	150.00	155.00	119.50	98.50	0.27
Blue Hyslop	144.00	149.00	108.00	---	--
Blue Norco	152.00	156.00	148.00	---	--
LSD.05	0.93	1.48	7.15	11.75	NS

NS = No significant difference between the means genetic marker lines the cultivars were intermediate for heading and anthesis dates. The difference between the mean values for both heading and anthesis dates of all the entries and the genetic marker lines were significant.

As observed in Table 2, the mean values for plant height were quite different depending on the entry. Jackmar was the shortest (105.5 cm). Stephens, SPN/AU/YMH and Hill 81 were intermediate while 55.1744/7C//SU/RDL and Yamhill, 119.5 cm and 125.5 cm tall respectively, were the tallest entries. The genetic marker line Blue Norco (148 cm) was significantly taller than all the entries. The mean values for plant heights of the entries and genetic marker lines were significantly different.

Additionally, the data in Table 2 showed that the mean number of tillers per linear meter ranged from 69.5 to 110.5. Yamhill had the lowest mean number of tillers per linear meter of 69.5. Apart from Stephens, all the other winter wheat cultivars and experimental lines had intermediate mean number of tillers per linear meter. Stephens had the highest mean number of tillers per linear meter of 110.5. Difference between values for mean tillers per linear meter of the winter wheat cultivars and the experimental lines were significant.

Percent Natural Outcrossing

Although the winter wheat cultivars and the experimental lines were close to genetic marker lines (20 cm apart), mean natural outcrossing was less than 1%. There were differences in the amount of natural outcrossing among the winter wheat cultivars and the experimental lines. Hill 81 had the highest mean value for natural outcrossing of 0.55% while the experimental line SPN/AU/YMH had the lowest mean value for outcrossing of 0.10%. All the other winter wheat cultivars and experimental line had intermediate mean values for the percent natural outcrossing. However, no significant difference between mean values for percent outcrossing of the entries grown in standard yield trial conditions were observed.

Study III

Agronomic Traits

The observed mean squares for the agronomic traits for different planting densities and cultivars are presented in Table 3. Observed mean squares did indicate that density and cultivar effects on the measured agronomic traits were significantly different. An Entry x Density interaction effect on days to heading and tillers per linear meter was also observed.

Data for agronomic traits in low and high seeding rates are presented in Tables 4 and 5. The observed mean values for agronomic traits in low and high seeding rates revealed that the entries headed and reached anthesis earlier in high seeding rate. Experimental line SPN/AU/YMH headed and reached anthesis four days earlier in high density than in low density. The mean difference in days to

Table 3. Observed mean square values for four agronomic traits involving six winter wheat entries when seeded at low and high densities at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Source of variation	Degrees of freedom	Days to heading	Days to anthesis	Plant height	Tillers per linear meter
Replication	3	0.39	0.94	50.08	163.13
Density	1	65.33**	60.75*	487.69**	1740.02*
Error a	3	2.94	5.58	10.52	182.58
Entry	5	68.45**	103.90**	303.87**	1844.29**
Error b	15	0.44	0.71	12.26	21.60
Entry x density	5	1.88**	1.05	25.54	53.32*
Error c	15	0.29	0.62	11.64	18.28
Total	47				

* = Significant F value at 5% probability level
** = Significant F value at 1% probability level

Table 4. Mean values for four agronomic traits involving four winter wheats, two experimental lines and two genetic marker lines seeded at low seeding rate at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Entry	Days to heading	Days to anthesis	Plant height (cm)	Tillers per linear meter
Stephens	145.75	149.50	99.00	100.25
Yamhill	147.00	151.25	114.25	67.75
Jackmar	147.00	151.00	100.75	77.50
Hill 81	150.25	154.50	102.50	71.50
SPN/AU/YMH	144.50	148.00	93.00	76.75
55.1744/7C//SU/RDL	151.50	157.00	107.50	97.00
Blue Hyslop	145.00	149.00	100.00	---
Blue Norco	152.00	157.00	153.00	---
LSD.05	0.76	1.07	4.52	4.52

Table 5. Mean values for four agronomic traits of four winter cultivars, two experimental lines and two genetic marker lines seeded at high density at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Entry	Days to heading	Days to anthesis	Plant height (cm)	Tillers per linear meter
Stephens	143.00	146.75	105.50	122.50
Yamhill	145.50	149.25	117.50	76.50
Jackmar	145.50	149.25	104.00	87.75
Hill 81	147.25	152.75	108.25	83.75
SPN/AU/YMH	140.75	144.50	106.00	86.75
55.1744/7C//SU/RDL	150.00	155.25	114.00	105.75
Blue Hyslop	145.00	149.00	106.00	--
Blue Norco	150.00	156.00	148.67	--
LSD.05	1.04	1.36	5.81	8.38

heading and anthesis in both seeding rates were significant.

Mean plant height values for all the entries were generally lower in the low density planting. For example, in low density planting the experimental line SPN/AU/YMH was 13 cm shorter than in high density planting.

Mean number of tillers per linear meter for all the cultivars and experimental lines were also less in the lower seeding rate. Stephens in high density planting averaged, 22 more tillers than in low density planting.

Mean Percent Natural Outcrossing

The observed mean square values for both low and high seeding rates are presented in Table 6. Mean square values revealed that natural outcrossing in combined seed rates were influenced by the replicates, the entries and distance. There was no significant interaction between the treatments.

For the six entries, the mean values of natural outcrossing for the combined seeding rates are presented in Table 7. For all entries, the observed mean natural outcrossing was less than 1%. Jackmar had the highest natural outcrossing of 0.16% followed by 55.1744/7C//SU/RDL with a value of 0.11%. Hill 81 and Stephens had intermediate values of 0.08% and 0.06% respectively. Yamhill and the experimental line SPN/AU/YMH had the lowest value for natural outcrossing of 0.03%.

As sampling distance increased from 0.5 m to 8 m away from the

Table 6. Observed mean square values for percent natural outcrossing of six winter wheat entries when seeded at low and high densities at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Source of variation	Degrees of freedom	Percent natural outcrossing
Replication	2	0.136**
Density	1	0.041
Distance	4	0.127**
Distance x Density	4	0.016
Error a	18	0.0194
Entries	5	0.075**
Entries x Density	5	0.018
Entries x Distance	20	0.006
Entries x Density x Distance	20	0.004
Error b	100	0.011
Total	179	

* = Significant F value at 5% probability level
** = Significant F value at 1% probability level

Table 7. Mean values for percent natural outcrossing involving four winter wheat cultivars and two experimental lines sampled at five different distances from the genetic marker lines at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Entry	DISTANCE					Entry mean
	0.5 m	1 m	2 m	4 m	8 m	
Stephens	0.14	0.08	0.04	0.03	0.003	0.06
Yamhill	0.09	0.05	0.01	0.02	0.00	0.03
Jackmar	0.32	0.16	0.17	0.10	0.05	0.16
Hill 81	0.17	0.13	0.08	0.04	0.01	0.08
SPN/AU/YMH	0.07	0.07	0.01	0.00	0.005	0.03
55.1744/7C//SU/RDL	0.23	0.12	0.12	0.05	0.03	0.11
Distance mean	0.17	0.10	0.07	0.04	0.02	

LSD .05; For testing entry means = 0.05
For testing distance means = 0.07

blue aleurone pollen source, the recorded mean natural outcrossing decreased from 0.17% to 0.02%. At a distance of 4 m, the recorded outcrossing of 0.04% was about one fourth of that recorded at 0.5 m (Table 7).

The relationship between sampling distances and percent natural outcrossing of the six entries is illustrated in Figure 2. The graphs show that all the entries had drastic drop in values of percent natural outcrossing as sampling distance increased from 0.5 m to both 1 m and 2 m. There was also another distinctive drop in values of outcrossing as sampling distance increased from 2 m to 4 m. However, there was little or no change in values of percent outcrossing between 4 m and 8 m for any of the entries.

Figure 2 also illustrates that entries such as Jackmar, 55.1744/7C//SU/RDL and Hill 81 maintained relatively high values of outcrossing in all sampling distances.

Correlations

Correlation coefficients measuring the degree of association between the means of measured variables (see Appendix Table 11) and mean percent natural outcrossing in Study III are presented in Table 8. All correlation coefficients values were positive and generally low. Plant height had the lowest correlation coefficient of 0.12 whereas wind velocity had the highest value of 0.70. These correlation coefficient values were not statistically significant.

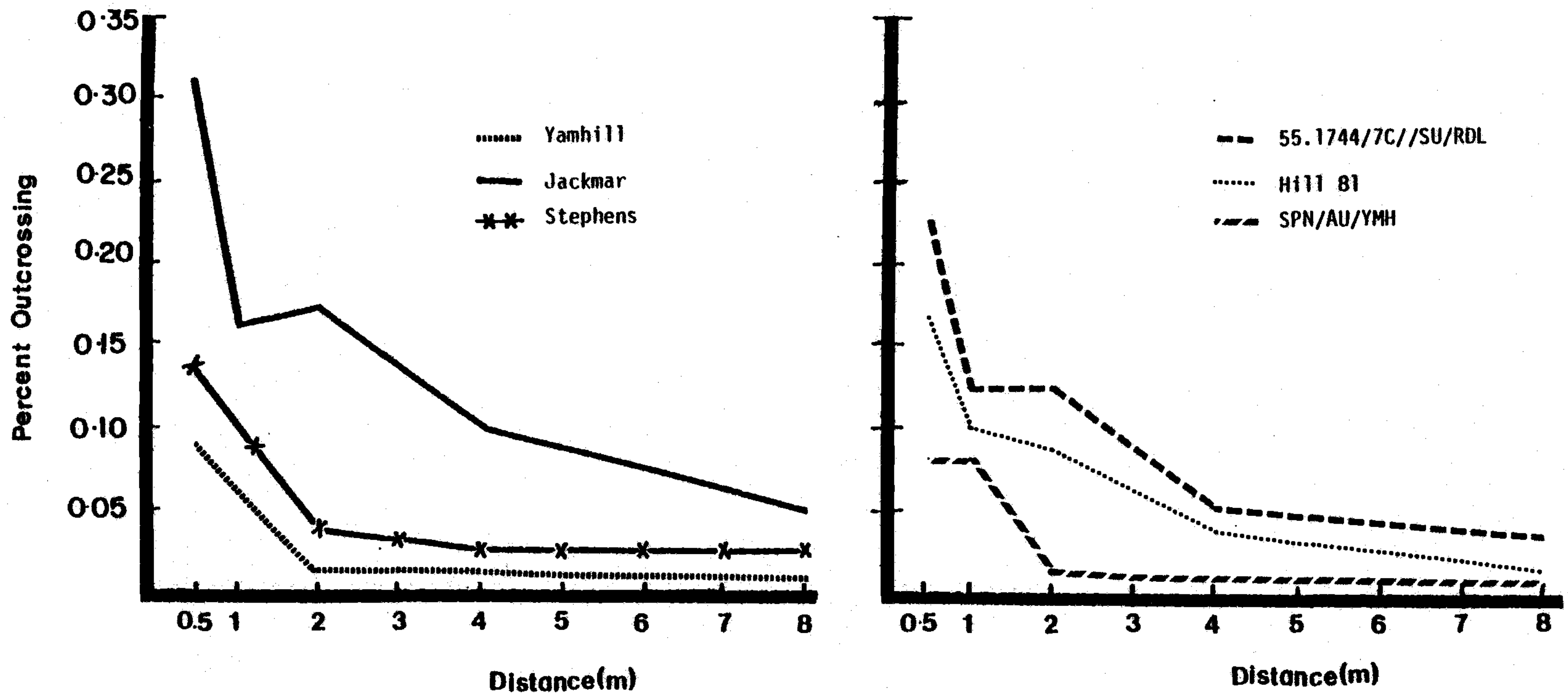

Figure 2. Relationship between the amount of outcrossing of four wheat cultivars and two experimental lines and the distance from blue aleurone pollen source.

Table 8. Correlation between mean percent outcrossing and means of four agronomic traits of wheat entries when seeded at low and high densities and wind velocity at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Trait	'r' value
Days to heading	+ 0.34 NS
Days to anthesis	+ 0.34 NS
Plant height	+ 0.12 NS
Tillers per linear meter	+ 0.14 NS
Wind velocity	+ 0.70 NS

n = 8
NS = Non significant

Regression

A simple regression coefficient was computed to determine how percent natural outcrossing changed with a given change in sampling distance. Relationship between the logarithmic amount of percent natural outcrossing and the distance from blue aleurone pollen source is presented in Figure 3. The simple regression equation showing the relationship between logarithmic percent natural outcrossing and the distance from the blue aleurone pollen source is expressed as follows:

$$\text{Log outcrossing} = -0.8325 - 0.1292 \times \text{distance (m)}$$

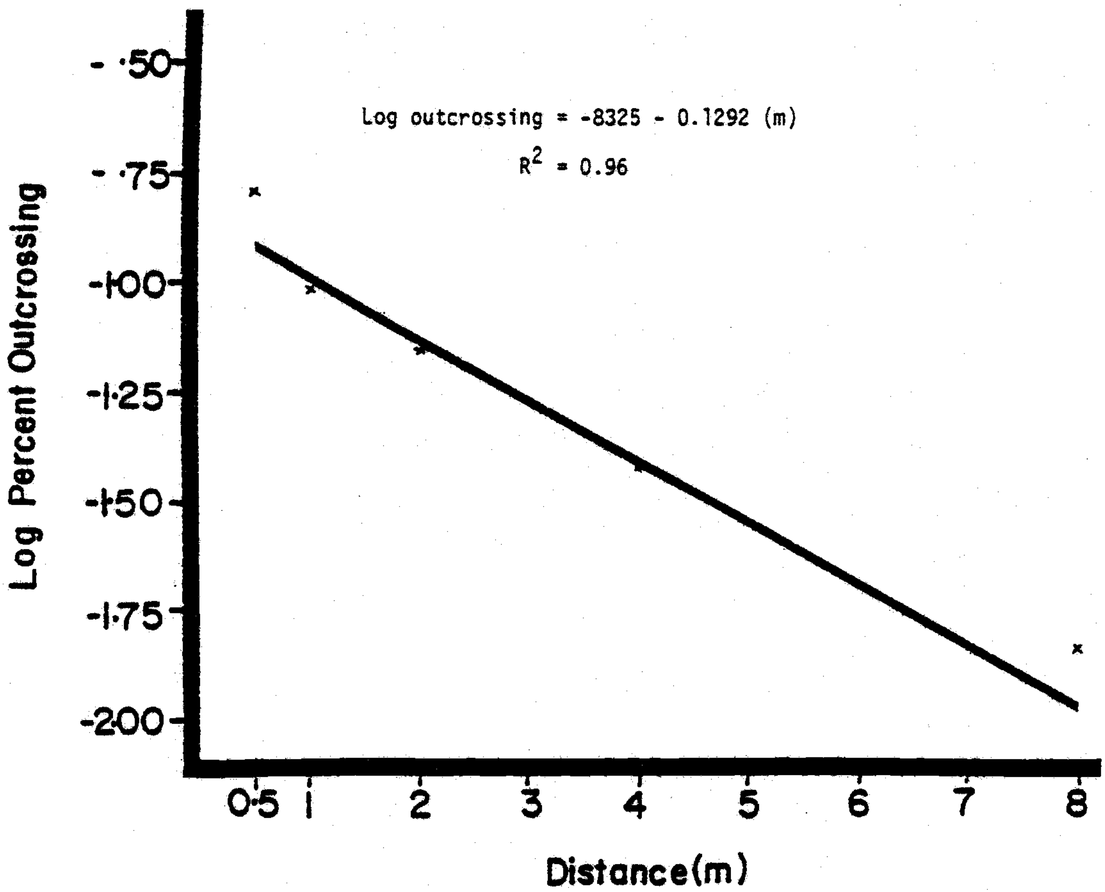

Figure 3. Relationship between logarithmic values for mean percent outcrossing (involving six wheat entries seeded at low and high densities) and the sampling distance.

DISCUSSION

A large investment in resources is required to develop a new wheat cultivar. This includes the expenditure of time by the breeder to say nothing of funds for land, supplies and laboratory facilities. Thus once such an effort is expended to develop a new cultivar with the desired genetic attributes, the genetic identity and integrity of this material must be maintained. Systems for multiplying and maintaining seed stocks have been established to ensure that the genetic properties of a new cultivar are maintained. Once a cultivar is released, the plant breeder provides detailed information regarding the characteristics and the pedigree of the new cultivar. Concerns have been expressed that perhaps during the selection and evaluation phases of the breeding program contamination can occur due to natural outcrossing. Thus the genetic properties and the pedigree assigned to a new cultivar may be incorrect. Additionally, concerns have been expressed that when seeds of self-pollinating species in germplasm collection centers are periodically revitalized, after several years of storage, they are grown so close to each other that outcrossing might occur and distort the genetic worth of the valuable germplasm. It was the purpose of this investigation to measure the extent of outcrossing occurring in various phases of a breeding program, and to determine if existing isolation distance for the multiplication of improved new cultivars of wheat is adequate.

The pedigree system, one of the most widely used methods of plant improvement, involves hybridization of parents with desirable attributes followed by selection of promising progenies in subsequent segregating generations. In using this method plants are spaced planted from F_2 through F_5. As homozygosity is reached in the F_6, evaluation of the selected progeny is continued by establishing replicated yield trials. These yield trials are solid seeded and may be conducted in many locations with varied environments. Evaluation of superior progeny is also done in regional yield trials with the most promising lines being eventually named and released as new varieties. Upon release, multiplication of seed stocks is done through a generation system which includes breeders, foundation, registered and certified seed. As the seed passes from one class to the next, the requirements regarding isolation distances and purity standards become less rigid.

The results of the investigation of natural outcrossing in spaced planted F_2 populations and solid seeded advanced generations of wheat will be discussed as they apply to: 1) efficiency of the method used to detect outcrossing, 2) association of agronomic traits with outcrossing, 3) relationship between environmental factors and outcrossing, and 4) effects of outcrossing on the developmental, maintenance and multiplication phases of a breeding program.

Efficiency of the Method Used to Detect Outcrossing

With the development of the genetic marker lines, Blue Norco and Blue Hyslop, it was possible to measure the actual outcrossing within the same year. Outcrossed seeds by expressing blue xenia on their aleurone layer could be identified on the basis of blue color at harvest time.

Late maturing Blue Norco and early maturing Blue Hyslop were mixed and planted at two separate dates in order to achieve floral synchronization with both the F_2 populations and solid seeded entries. The second planting date of the blue aleurone lines had poor emergence as the fields were extensively flooded in late November 1981. Consequently the pollen load of these lines relative to that of wheat cultivars and the experimental lines in high density planting (100 kg/ha) was low. Moreover, Blue Hyslop being shorter probably did not contribute as much pollen to be dispersed by the wind.

Furthermore, there was no way of detecting outcrossing caused by foreign pollen which carried no genetic marker genes. Any male sterile floret had a chance of receiving pollen from fertile florets of either the same cultivars or other cultivars within the experimental field and not necessarily from blue aleurone marker lines per se. Thus blue aleurone pollen source as a method of detecting outcrossing perhaps gave a conservative estimate (less than 1% for the studies in this experiment). However, it is quick, less cumbersome and cheap even though not entirely effective in measuring absolute amount of natural outcrossing. Other methods such as electrophoresis, phenol reaction and other chemical tests, which are expensive and time consuming, might have detected even higher natural outcrossing rates.

Association of Cultivar Agronomic Traits with Natural Outcrossing

Although differences in mean number of days to heading and to anthesis in all the studies were statistically significant, flowering duration of all experimental materials occurred within an interval of 10 days. This was in agreement with findings of Patterson (1970), De Vries (1971) and Balla (1980). The findings also confirm Allan's (1980) comment that wheat at high latitudes head at nearly the same time and consequently provide an excellent opportunity for natural outcrossing.

Findings of Beri and Anand (1970) suggests that taller wheat plants would have less outcrossing as they shed more pollen into the air, thereby creating a cloud or barrier of their own pollen. However, the results from this study do not support their findings. The tallest experimental line, 55.1744/7C/SU//RDL and the cultivars Jackmar and Hill 81, which were intermediate in height, had high percentage natural outcrossing (based on the mean values) when solid seeded. Degree of floral synchronization seems to have been more important in determining amount and extent of natural outcrossing than relative plant heights per se. For instance, experimental line SPN/AU/YMH, headed and reached anthesis much earlier than the genetic marker lines and had the lowest value of percent natural outcrossing in solid seeded conditions.

Tillering in wheat extends flowering periods. Wheat spaced planted at 30 cm x 60 cm, produces as many as 100 tillers. A flowering duration of 14 to 21 days is consequently achieved due to the secondary tillers (Allan, 1980).

Although, the number of tillers per plant in F_2 populations in this study were variable, the overall population means were not significantly different. Late tillers may have been instrumental in bringing about outcrossing in F_2 populations such as SDY/HER and STP/YR//BUC which headed and reached anthesis before the marker lines.

In solid seeded winter wheat cultivars and experimental lines, the seeding rate of 100 kg/ha, resulted in more tillers per linear meter than the low seeding rate of 25 kg/ha. This was more of a reflection of seeding rate rather than the ability of a cultivar to produce tillers. Density x Entry interaction was significant for the number of tillers per linear meter; however, this was not translated into a significant effect on mean percent natural outcrossing. In general, low density planting had a higher percent outcrossing than high density planting. This seems to have been due to relatively higher pollen load of genetic marker lines in relation to the pollen loads produced by the entries at the low density planting rather than relative number of tillers in the two planting densities.

With regard to spike density, Jackmar, which has a compact spike, had relatively high percent natural outcrossing. In contrast, Yamhill which has an extended dense awnless spike and reached anthesis nearly at the same time as Jackmar, had a very low value of percent natural outcrossing. Experimental line 55.1744/7C//SU/RDL has a lax spike, but its percent natural outcrossing was significantly lower than Jackmar. Apparently, in this study, spike density per se did not influence the extent of percent natural outcrossing recorded.

Association of Environmental Factors with Natural Outcrossing

Extreme environmental factors have adverse effects on pollen development and release (Frankel and Galun, 1977). An examination of meterological data (see Appendix Tables 2 and 3) for Hyslop Experimental Farm, reveals that extremes in temperatures such as those cited by Suneson (1937) did not occur. In the months of May and June 1982, when anthesis was occurring, the relative humidity was moderate, solar radiation was high and wind speeds reached 16 km/hr. Rainfall recorded at this period was only 40 mm.

According to De Vries (1972) most pollen is shed at relative humidity of between 70 and 75%. Flowering pattern in wheat is also reported to be diurnal with two bimodal peaks at hours 08.00 to 11.00 and 15.00 to 18.00 (Patterson, 1970 and De Vries, 1971). The mean relative humidity at the biomodal peaks, at Hyslop Farm was 54% and 43% respectively (Appendix Table 2). These figures tend to suggest that relative humidity was not ideal for maximum pollen shed during the flowering period.

In this experiment wind speed appeared to be a major factor influencing the extent of natural outcrossing. Viability of wheat pollen in the air is only approximately 5 minutes when environmental effects are adverse (Arp, 1967 and Curtis and Johnston, 1969). However, at wind speed of 3 km/hr pollen would be dispersed and carried to a distance of 100 m in 2 minutes. It would appear in this study that wind was instrumental in disseminating marker pollen away from its source. Other graduate students working at approximately 40 m, in the easterly direction of the field where this experiment was conducted reported the presence of blue seeds (outcrossed) in their experimental material. Wind, therefore, can carry wheat pollen to great distances. This is in agreement with the findings of Wilson (1968) and Yeung and Larter (1972).

A significant distance effect on percent natural outcrossing existed in both seeding rates. The further the sampling distances were from the blue aleurone pollen source the less the amount of natural outcrossing obtained. As sampling distance increased from 0.5 m to 8 m away from the blue aleurone pollen source the recorded mean natural outcrossing decreased from 0.17% to 0.02% (see Table 7 and Figure 2). This was in agreement with the findings of Jones and Newell (1946) and Bateman (1947) that both foreign pollen load and contamination of seed crops decrease with increasing distance from the foreign pollen source. Results from the study do indicate that even at the maximum sampling distance of 8 m, outcrossing was still being observed.

In summary, natural outcrossing in wheat is associated largely with: 1) varietal factors such as heading and anthesis dates and proper synchronization of flowering periods of many cultivars, and 2) environmental conditions such as adequate wind speeds. Factors such as plant height, number of tillers per plant (per linear meter) and planting densities had less effects on extent of natural outcrossing.

Effects of Natural Outcrossing on Developmental, Maintenance and Multiplication Phases of a Breeding Program

The breeding of improved cultivars and their subsequent multiplication is achieved by isolation. This can be either in time or in space (distance). Isolating wheat cultivars in time is unrealistic since wheat at high latitudes head at nearly the same time (Allan 1980). Breeders and certification personnel give different emphasis to isolation. The major concerns for the breeders are twofold: 1) isolate parents so as to execute a planned and predetermined cross and 2) isolate superior germ plasm so as to maintain their genetic purity and identity throughout the developmental phase. On the other hand, certification personnel are concerned primarily with maintaining genetic purity of improved cultivars. Adequate isolation distances in both cases are based on mode of pollination of the cultivar being handled. If natural outcrossing is relatively high in self pollinating cereals a breeder should be concerned about growing: 1) a disease spreader row in close proximity to superior lines, 2) several lines of superior parents together in a crossing block, and 3) F_2 or other segregating generations of different populations in close proximity to each other. In a certification program one would also want to establish adequate isolation distances to avoid possible outcrossing.

In this study, natural outcrossing was observed in: 1) spaced planted F_2 populations, and 2) advanced generations of wheat grown in both yield trial and seed increase conditions. In breeding programs of wheat involving pedigree system of plant improvement, cases are known where a red seeded line has resulted from a white seeded parental pedigree and vice versa. Such an occurrence suggests that outcrossed plants are selected and advanced to subsequent generations in a breeding program. However, if outcrossing occurs between either two white seeded lines or two red seeded lines, one would not be able to detect the outcrossed seed based on seed color. In such instances cultivars may eventually be released with incorrect pedigree.

In a breeding program the seeds used to plant yield trials and crossing blocks are threshed singly and examined for uniformity of seed attributes. After that they are grown in either single head or plant rows and examined for their trueness to type. From these handling procedures effects of natural outcrossing will be minimized while handling both breeding stock and the improved genotypes in a breeding program so long as extremes in environmental conditions are not encountered.

While multiplying seed of new cultivars isolation distance imposed by certifying agency in Oregon is 27 m for foundation seed and 2 m for both registered and certified generations. In this study outcrossed seeds were found as far as 40 m away from blue aleurone pollen source. Isolation distance of 27 m for multiplication of foundation seed may be adequate but the isolation distance of 2 m for the multiplication of both the registered and certified generations of wheat seed is inadequate. In this study different cultivars and experimental lines showed different pattern and extent of outcrossing (Figure 2) while seeded in standard yield trial conditions. Therefore, isolation distance will depend on the cultivar being multiplied.

In germplasm collection centers, where small populations of self pollinating species are grown side by side during revitalization of seed viability of the germplasm, outcrossing may distort and alter the genetic identity and integrity of valuable stocks of germplasm.

Suggestions and Recommendations

Protection against foreign pollen in developmental and multiplication phases of a breeding program is nearly impossible. Roguing and purification processes such as the use of head rows or phenol reaction as a test for trueness to type will always have to be practiced to minimize possibility of accidental mixtures due to outcrossing. These procedures merely help the plant breeders to eliminate outcrossed plants or seed but do not prevent outcrossing. From a practical point of view, plant breeders will have to accept presence of a certain amount of outcrossed plants in their breeding programs.

A careful examination of the trend of natural outcrossing in respect to distance from the blue aleurone pollen source in this study (Figure 2 and Appendix Figure 1) suggest that an isolation distance of between 4 and 8 m would be adequate for production of registered and certified wheat seed. However, data available in the literature on isolation distances in production of seed crops in self pollinating cereals are scarce. There is, therefore, a need to generate more data under many varied environmental conditions, before any recommendation can be made. Results of this investigation suggest that current isolation requirements of 2 m may not be adequate for such cultivars as Jackmar and Hill 81.

In collection centers where germplasm is being preserved, it would be desirable to adequately isolate stocks either in space (where land and resources are available) or by growing barrier lines of a different species (e.g. barley) in order to maintain genetic integrity of each individual accession number.

SUMMARY AND CONCLUSION

The major objective of this study was to measure the level of outcrossing in various stages of a wheat breeding program. This included spaced planted F_2 populations and advanced generations of winter wheat when solid seeded at high and low seeding rates. A further objective was to determine if the established distances for multiplication of wheat seed are adequate under the existing certification requirements.

Four different F_2 populations, four wheat cultivars and two experimental lines were selected as experimental wheat materials. To determine the amount of outcrossing two marker lines which carry genetic factors for blue aleurone color were used.

The results of this investigation can be summarized as follows:

1) Levels of outcrossing recorded on mean basis were less than 1% in all experiments. However, even this apparent low level of outcrossing should be of concern to those engaged in breeding and certification programs and in germplasm collection and preservation.

2) From a practical consideration breeders may have to accept the presence of outcrossing and perhaps incorrect pedigree listing in their programs. However, in early generation material it may be desirable to plant barley or some other species between different segregating populations to reduce the chance of outcrossing.

3) The established minimum isolation distance of 27 m for the multiplication and production of foundation seed in Oregon seems

to be adequate. If high levels of genetic purity are to be assured, the current isolation distance of 2 m should be increased to 4 m for registered and certified wheat seed when grown in the Willamette Valley. There is need for further studies regarding the effective isolation distances for maintaining genetic purity when multiplying wheat under different environmental conditions.

4) When seed viability is being revitalized in germplasm collection centers, it may be desirable to separate the wheat accessions by a barrier of barley or some other plant species.

5) Major factors favoring outcrossing were wind velocity, heading and anthesis dates and synchronization of flowering periods among cultivars.

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APPENDICES

Appendix Table 1. Specific characteristics of the cultivars, experimental lines, genetic marker lines, and parents of the F_2 populations studied.

STEPHENS: Nord Desprez/Pullman 101 OR 65-116-70-MBW2

Stephens is a semi-dwarf, soft white winter wheat which was developed and released by Oregon Agricultural Experiment Station in cooperation with SEA,USDA. The spike is awned, white chaffed, lax and semi-erect. Stephens is adapted to the winter wheat growing areas of the Pacific Northwest and expresses superior yield potential under low and high rainfall (also under irrigated) conditions. It is medium in maturity.

YAMHILL: Heines VII-Redmond (Alba)

Yamhill, a soft white winter wheat, was developed and released by Oregon State University. It is mid-tall and late in maturity. The spike is awnless, white chaffed, erect and has compact spikelets. Yamhill is adapted to high rainfall conditions.

JACKMAR:

Jackmar is a soft white club wheat, which was selected from a field of cultivar 'Omar' (Elgin 19/Elmar). It is medium in height and late in maturity. The spike is awnless, white chaffed, short, erect and has compact spikelets. Jackmar is adapted to low and intermediate rainfall areas.

HILL 81: Yamhill/Hyslop

Hill 81 is a soft white winter wheat; it is mid-tall and mid-season in heading and has white stiff straw. The spike is awned, mid-dense and inclined. It is widely adapted to the winter wheat growing areas of the Pacific Northwest. Hill 81 was developed and released by Oregon State University.

SPN/AU/YMH:

A soft white, mid-tall experimental line. It is medium to early in maturity. The spike is awnless, white chaffed and has compact spikelets. This experimental line has a portion of rye chromosome.

55.1744/7C//SU/RDL:

A hard red mid-tall experimental line. It is late in maturity. The spike is awned, white chaffed, semi-lax and long.

SDY/HER:

A F_2 which resulted from a cross of 'Sturdy' and 'Hermosillo'.

Sturdy is a release of Texas A and M University. It is hard red winter wheat which is semi-dwarf and early in maturity. The spike is awned, white chaffed and semi-lax. It is adapted to the central and southern areas of the Great Plains of USA. It does well under low and intermediate rainfall conditions.

Hermosillo is a hard red spring wheat which was released by INIA, Mexico. It is semi-dwarf and very early in maturity. The spike is awned, white chaffed and semi-lax. It is adapted to all spring wheat growing areas and does well under irrigated and rainfed conditions.

STP/YR//BUC:

A F_2 which resulted from a three-way cross of 'Strampelli', 'Yecora 76' and 'Buckbuck'.

Strampelli is a hard red winter (facultative) wheat which is grown in Italy and high plateau regions of North Africa. It is semi-dwarf and early in maturity. The spike is awned, white chaffed and semi-lax.

Yecora 76 is a hard white spring wheat which was released by INIA, Mexico. It is semi-dwarf and early in maturity. The spike is awned, white chaffed and erect. It has high yield potential and is adapted widely across spring wheat growing areas.

Buckbuck is a hard red spring wheat, which was released by CIMMYT as a breeding line. It is semi-dwarf and medium in maturity. The spike is awned white chaffed and semi-erect. It has high yield potential and is adapted to areas with high fertility and either high or intermediate rainfall.

MHB/VEE:

A F_2 which resulted from a cross of 'Marris Hobbit' and 'Veery'.

Marris Hobbit is a soft red winter wheat which was developed and released by Cambridge, England. It is dwarf and late in maturity. The spike is awnless, white chaffed, semi-erect and has compact spikelets. It is adapted to high rainfall areas (also irrigated conditions). It has high yield potential and a long growing cycle.

Veery is a spring x winter cross with facultative potential. Several new varieties are being increased (or already under commercialization) from this Veery cross. Veery is a CIMMYT breeding line which is semi-dwarf and intermediate in maturity. The spike is awned, white chaffed and semi-erect. It is adapted widely over many spring wheat growing areas of the world.

VORO/ASP:

A F_2 which resulted from a cross of 'Aspen' and 'Vorochilovskaja VB 4-279-2'.

Aspen is a hard red winter wheat used in the crossing block of Oregon State University. It is semi-dwarf and intermediate in maturity. The spike is awned, white chaffed and semi-erect. It is adapted to intermediate winter environments.

Vorochilovskaja VB 4-279-2 is a hard red winter wheat which is grown commercially in Russia. It is tall and very early in maturity. The spike is awned, white chaffed and erect. It is adapted to low and intermediate rainfall areas.

BLUE HYSLOP: Agropyron/Baart/Hyslop[1]

A genetic marker line which was developed by USDA scientists at Oregon State University. It has a portion of the chromosome of Agropyron which conditions the dominant blue xenia effects on outcrossed seeds. Blue Hyslop is semi-dwarf and medium in maturity. The spike is large awned and mid-dense.

BLUE NORCO: Agropyron/Baart/Norco[1]

A genetic marker line which was developed by USDA scientists at Oregon State University. Like Blue Hyslop, it also has a portion of the chromosome of Agropyron which was backcrossed into it from blue Baart to obtain the blue aleurone color. Blue Norco is tall and late in maturity. The spike is mid-dense, short and awned.

Appendix Table 2: Summary of meterological data at Hyslop Experiment Farm. Corvallis, Oregon (1981-82).

Month	Temperature (C°) Max.	Temperature (C°) Min.	Humidity (%) Max.	Humidity (%) Min.	Precipitation (mm)	Evaporation (mm)	Radiation ($Cal/cm^{-2}day^{-1}$)
September	25.1	8.5	98.4	38.8	78.5	139.3	347
October	16.6	4.9	99.0	60.5	140.2	48.4	202
November	12.4	4.3	99.0	71.0	170.9	---	105
December	9.3	2.8	99.0	79.5	355.1	---	64
January	6.1	0.4	99.0	82.7	183.1	---	84
February	9.6	1.6	93.6	61.3	180.8	---	154
March	12.4	2.0	99.0	53.2	89.9	---	265
April	14.3	2.4	96.8	46.1	116.1	85.1	376
May	20.1	5.9	98.4	37.0	12.5	151.9	483
June	23.5	10.6	97.6	44.7	38.4	145.8	417
July	25.4	10.8	98.0	38.7	10.9	176.0	444
August	27.2	10.8	97.5	34.1	7.1	168.1	357

Snow was recorded in the months of December, January and February.
In May mean relative humidity at 10:00 a.m. and 4:00 p.m. was 55% and 44% respectively.
In June mean relative humidity at 10:00 a.m. and 4:00 p.m. was 53% and 39% respectively.

Appendix Table 3. Summary of hourly wind velocity (km/hr) at Hyslop Experiment Farm. Corvallis, Oregon (15 May to 15 June 1982).

Time	Wind Velocity								Mean hourly speed
	North	East	South	West	North East	South East	South West	North West	
6- 7 a.m.	9.0	S	4.8	S	3.2	S	1.6	S	3.7
7- 8 a.m.	8.0	S	4.8	1.6	S	3.2	1.6	16.0	5.9
8- 9 a.m.	11.6	S	6.8	S	4.8	2.7	4.8	14.5	7.5
9-10 a.m.	11.8	S	5.5	S	3.2	1.6	4.0	13.7	6.6
10-11 a.m.	10.5	S	6.4	9.7	9.0	S	8.0	9.7	8.9
11-12 noon	11.7	S	S	S	9.7	S	7.6	8.8	9.4
12- 1 p.m.	10.6	S	4.0	S	10.0	S	7.6	14.0	9.2
1- 2 p.m.	9.2	S	S	12.9	9.0	3.2	4.2	14.1	8.8
2- 3 p.m.	10.5	S	S	12.9	14.5	1.6	6.0	14.5	10.0
3- 4 p.m.	11.8	S	S	12.9	15.3	1.6	6.8	11.7	10.0
4- 5 p.m.	9.7	S	S	10.4	7.5	S	12.9	15.1	11.1
5- 6 p.m.	12.2	S	S	10.9	14.5	S	10.9	9.7	11.6

The anemometer in use at Hyslop Weather Station does not pick up wind velocities less than 1.6 km/hr. Hence, wind velocities less than 1.6 km/hr are designated 'S' (slight air movement).

Appendix Table 4. Observed mean square values for four agronomic traits and percent outcrossing involving four F_2 populations spaced planted at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Source of Variation	Degrees of freedom	Days to heading	Days to anthesis	Plant height	Tillers per plant	Percent outcrossing per plant	Percent outcrossing per population
Replication	3	4.17	3.56	60.81	6.23	0.027	77.23
Cross	3	95.33**	88.73***	357.54***	6.06	0.088**	998.23**
Error	9	3.72	2.01	17.37	3.12	0.011	104.40
Total	15						

Cross = The four F_2 populations used in the study

** = Significant F value at 1% probability level

*** = Significant F value at 0.1% probability level

Appendix Table 5. Observed mean square values for four agronomic traits involving six wheat entries when seeded at standard yield trial conditions at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Source of Variation	Degrees of freedom	Days to heading	Days to anthesis	Plant height	Tillers per linear meter	Percent natural outcrossing
Replication	1	0.33	0.33	5.33	140*	0.0014
Entry	5	18.73***	28.13***	129.60**	432**	0.046
Error	5	0.13	0.33	7.73	20.88	0.019
Total	11					

* = Significant F value at 5% probability level

** = Significant F value at 1% probability level

*** = Significant F value at 0.1% probability level

Entry = four winter wheat and two experimental lines

Appendix Table 6. Observed mean square values for four agronomic traits involving six wheat entries when seeded at low density at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Source of variation	Degrees of freedom	Days to heading	Days to anthesis	Plant height	Tillers per linear meter
Replications	3	1.89**	2.38*	51.44**	53.38*
Entry	5	28.77**	43.98**	214.37**	735.14**
Error	15	0.26	0.51	9.01	8.94
Total	23				

* = Significant F value at 5% level of probability
** = Significant F value at 1% level of probability

Appendix Table 7. Observed mean square values for four agronomic traits involving six wheat entries when seeded at high density at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Source of variation	Degrees of freedom	Days to heading	Days to anthesis	Plant height	Tillers per linear meter
Replications	3	1.44	4.15*	9.15	302.00**
Entry	5	47.57**	60.98**	115.04**	1162.47**
Error	15	0.48	0.82	14.89	30.93
Total	23				

* = Significant F value at 5% level of probability
** = Significant F value at 1% level of probability

Appendix Table 8. Analysis of variance table for percent natural outcrossing (combined low and high planting densities) involving six wheat entries at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Source of variation		Degrees of freedom		Mean squares		F
Replication			2		0.136	7.01**
Density			1		0.041	2.11
Distance			4		0.127	6.55**
Distance x Density			4		0.016	0.82
Replication x Density	Error a	2	18	0.035	0.0194	
Replication x Distance		8		0.023		
Replication x Distance x Density		8		0.012		
Entry			5		0.075	6.82**
Entry x Density			5		0.018	1.64
Entry x Distance			20		0.006	0.55
Entry x Distance x Density			20		0.004	0.36
Replication x Entry	Error b	10	100	0.028	0.011	
Replication x Density		10		0.011		
Replication x Distance		40		0.012		
Replication x Distance x Density x Entry		40		0.006		

** = Significant F value at 1% probability level

Appendix Table 9. Mean values for percent natural outcrossing involving six wheat entries sampled at five distances in low density planting at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Entry	DISTANCE					Entry mean
	0.5 m	1 m	2 m	4 m	8 m	
Stephens	0.210	0.067	0.050	0.023	0.000	0.070
Yamhill	0.093	0.047	0.020	0.040	0.000	0.040
Jackmar	0.480	0.240	0.200	0.147	0.053	0.224
Hill 81	0.220	0.090	0.100	0.030	0.007	0.089
SPN/AU/YMH	0.077	0.053	0.000	0.000	0.000	0.026
55.1744/7C//SU/RDL	0.243	0.143	0.080	0.060	0.027	0.111
Distance mean	0.221	0.107	0.075	0.050	0.014	

Appendix Table 10. Mean values for percent natural outcrossing involving six wheat entries sampled at five distances in high density planting at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Entry	DISTANCE					Entry mean
	0.5 m	1 m	2 m	4 m	8 m	
Stephens	0.067	0.097	0.020	0.027	0.007	0.043
Yamhill	0.080	0.050	0.000	0.000	0.000	0.026
Jackmar	0.167	0.080	0.143	0.060	0.037	0.097
Hill 81	0.113	0.117	0.050	0.040	0.020	0.068
SPN/AU/YMH	0.060	0.083	0.027	0.000	0.010	0.035
55.1744/7C//SU/RDL	0.210	0.103	0.163	0.037	0.023	0.107
Distance mean	0.116	0.088	0.067	0.027	0.016	

Appendix Figure 1. The relationship between mean percent natural outcrossing and the distance from blue aleurone pollen source. (Data from Table 7)

Appendix Table 11. Data used to compute the correlation coefficient values between percent natural outcrossing and four agronomic traits involving six wheat entries when seeded at low and high densities and wind velocity at Hyslop Experimental Farm, Corvallis, Oregon (1981-82).

Percent natural outcrossing	Days to heading	Days to anthesis	Plant height (cm)	Tillers per linear meter	Wind velocity
0.047	145.65	150.53	110.33	98.33	4.10
0.022	145.83	150.17	107.67	83.17	2.70
0.090	145.00	149.00	110.17	101.83	8.60
0.052	144.83	148.83	108.50	87.00	8.40
0.035	147.83	151.67	103.83	77.33	4.10
0.038	146.83	151.17	99.50	81.33	2.70
0.173	148.00	152.33	106.33	83.83	8.60
0.072	148.00	152.00	101.67	84.00	8.40