Pollination Biology and Determination of Yield in Buckwheat (*Fagopyrum esculentum* Moench.)

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of
Graduate Studies
by

Kedar N. Adhikari

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ABSTRACT

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Major Professor, Dr. Clayton G. Campbell.

There has been little improvement in the yield of buckwheat in comparison to cereal crops over the past several decades. Although there is a good market potential for buckwheat, traditional buckwheat growers have been shifting to other crops owing to its low productivity. Therefore, improvement in yield is a major challenge to the buckwheat breeders. The present study was undertaken to gain a better understanding on the pollination biology and the relationships of grain yield with some important parameters affecting yield in buckwheat.

An in vitro pollen germination technique was developed to assess the longevity of buckwheat pollen. The pollen viability, as measured by germination percentage by both in vitro and in vivo methods, was mainly affected by temperature within six hours after the first light. Maximum pollen germination was found two hours and six hours after the first light when the plants were maintained at 25 C and 20 C, respectively.

In the second experiment, the extent of natural outcrossing in buckwheat was investigated by utilizing a

monogenetically inherited recessive semi-dwarf character. Approximately 50 per cent outcrossing occurred where the semi-dwarf plants were immediately next to the exotic pollen source. Although outcrossing percentage decreased with increasing distance for the first few meters, it persisted throughout the experimental range of 100 m. No significant difference was found after 9 m distance from the pollen source. The sharp reduction in pollen flow within 3 m distance and the lack of directional influence on outcrossing, suggested that wind was not a major factor in the dispersal of buckwheat pollen. The intermittent low and high frequencies of outcrossing suggested that the intensity of natural outcrossing was not only a function of the distance between the two genotypes, but also a function of the foraging behavior of the pollinators. An isolation distance of 100 m was found to be not adequate to prevent inter-varietal pollination in buckwheat.

In the third experiment, ten buckwheat germplasms from diverse origins were evaluated for two years to elucidate the determination of yield . The primary components contributing to buckwheat yield were found to be plant stand, the number of seeds per inflorescence and the number of seeds per plant. The correlations of yield components with other agronomic traits and grain yield were found to be low and inconsistent owing to the high degree of environmental influence. However,

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1. GENERAL INTRODUCTION

Buckwheat is generally grown in the temperate areas of the world where growing seasons are short. It is primarily used as a grain crop for human consumption and feed for livestock and poultry. It is also used as a green manure crop, a smother crop to crowd out weeds and as a source of buckwheat honey. Buckwheat grain is an excellent source of protein due to a favorable amino acids composition. It is especially high in lysine, which has been found to be low in cereals. In order to meet the increasing food demand of the world population, efforts must be made to improve the productivity of less developed crops such as buckwheat.

Despite its short growing season, protein-rich grain and adaptation to low fertility conditions, the traditional buckwheat growers have shifted to other crops due to the low productivity. Buckwheat was a common farm crop in the temperate areas of North America, but now, it is of minor importance (Marshall, 1980). Its production has been decreasing in the Commonwealth of Independent States (CIS), which is the largest producer of buckwheat and in France, Japan and the United States. Buckwheat production in Canada declined steadily until the early 1960s, increased until the 1980s and has remained fairly constant since then (Campbell, 1992).

In the past decade, the area under buckwheat in Canada has remained at nearly 39,000 hectare with Manitoba accounting for three quarters of this area (Gubbels and Campbell, 1990). Approximately one-third of the crop is consumed locally and the rest is exported. The major importer of Canadian buckwheat is Japan accounting for approximately five million dollars worth of buckwheat annually (Campbell, personal communication).

There has been little effort invested in the improvement of this crop in comparison to many others, possibly due to the presence of a heteromorphic sporophytic self-incompatibility system (Marshall, 1980). As a result, many phenomena in its life cycle are still unknown. The severe inbreeding depression that occurs in the first few generations of selfing has prevented the use of a heterotic hybrid approach to yield improvement (Marshall, 1979; Campbell, 1992). If the number of flowers produced on a plant is an indicator of potential seed yield, buckwheat has a large unrealized seed yield. However, less than 12 per cent of the flowers produce mature seeds. The high seed abortion problem, which can occur at any period from post fertilization to complete seed development, is not yet understood.

Inadequate pollination and fertilization associated with self incompatibility, indeterminate growth habit leading to

profuse branching, moisture stress and high temperature at blossoming, and inefficient distribution of assimilate to the developing grains have been described as main causes of low seed yield in buckwheat (Sugawara, 1960; Kreft, 1983; 1986; Naṃai, 1990; Ruszkowski, 1990; Fesenko, 1990). The objectives of this research were 1.) to determine whether the pollen produced is viable and the effect of temperature and time on its viability, 2.) to investigate the extent of natural outcrossing and 3.) to elucidate the interrelationships between yield and yield related parameters in buckwheat.

2. GENERAL LITERATURE REVIEW

2.1. Origin

The genus Fagopyrum was thought to have been originated in central Asia and wild species are found in Manchuria and Siberia (Morris, 1947). Some authors have suggested its origin in the Himalayan region of either western China or northern India (Tahir and Farooq, 1988). Still other claims that ancestral home of buckwheat was the mountainous region of south-western China near the Himalayan region called as 'Arc Center' (Nakao, 1960 as cited by Ujihara, 1983). This has been confirmed by a recent discovery by Ohnishi (1991). He found a closely related wild species of F. esculentum in the Yunan province and postulated that F. esculentum had evolved from this species, and not from F. cymosum as had been thought

earlier (Campbell, 1976). Based on isozyme analysis, F. cymosum as the ancestral progenitor of cultivated buckwheat, had already been questioned as it was only distantly related to tartary and common buckwheat (Ohnishi, 1983). The cultivated species have 2n = 2x = 16 chromosomes, but both diploid and tetraploid forms have been recorded in the wild species F. cymosum (Ohnishi, 1986).

2.2. Taxonomy

Buckwheat, Fagopyrum esculentum Moench., belongs to the Polygonaceae family. Although four species had originally been described as cultivated under the genus Fagopyrum, Ohnishi (1986) has claimed them as only two: Fagopyrum esculentum (common buckwheat), and F. tataricum (tartary buckwheat). Munshi (1982) proposed that a form of F. tataricum, mostly distributed in the Kashmir region, should be nominclated as F. kashmirianum. However, Ohnishi (1986) could not find any isozyme variation between these two species and regarded them as the same species with different morphological characteristics. F. esculentum is the most commonly cultivated species of buckwheat followed by F. tataricum. F. esculentum and F. cymosum are self incompatible and require cross pollination by various insects, but F. tataricum is self compatible and self pollinated. None of these species are inter-crossable. Throughout this paper, buckwheat refers to

F. esculentum unless stated otherwise.

2.3. Adaptation

Buckwheat is a minor crop grown in temperate areas, mostly in the CIS, Poland, Canada, Japan, Brazil, France and Himalayan region of Nepal, China and India where the growing seasons are short. Although it is a temperate crop, it is very sensitive to frost. A temperature of -2 C can kill all the flower buds, flowers and leaves and can cause severe injury to the stem (Krotov, 1963). The resistance to low temperature increases during ontogenesis, but the reverse is true for the recuperating ability (Lachonov et al, 1989). Approximately 50 per cent of plants were killed and the rest were heavily damaged when exposed to a temperature of 2-3 C for a week at the time of germination, but most of the plants were undamaged at 5 C after the same treatment at flowering stage. Variability in frost resistance among genotypes was observed, but in a very narrow range (Lachonov et al, 1989).

Moisture requirement for buckwheat is high because of its large canopy, the absence of waxy layer and hairs on the leaves, a single layered epidermis on leaf surface and the large number of stomata on both sides of the leaves (Krotov, 1963). The shallow and poorly developed root system accounts for approximately 20 per cent of the root mass of cereals

(Krotov, 1963), while the coefficient of transpiration is 2-3 times as high as that of cereals (Krotov, 1963; Fesenko, 1990). Buckwheat is very sensitive to moisture stress, especially at the time of blossoming and fruiting. A one week dry period can kill all the flowers and buds (Lachonov et al, 1989). Normally, 89 per cent of the total moisture requirement is utilized from flowering to maturity and only 11 per cent from emergence to flowering (krotov, 1963).

Buckwheat is normally a day neutral plant, but genotypes from China, Nepal, India and Japan show a short day reaction. Ujihara (1983) classified agroecotypes in Japan based on long day sensitivity as insensitive (summer type), sensitive (autumn type) and intermediately sensitive (intermediate type). Buckwheat has a very short grain filling period of 21-24 days as compared to 20-40 days in wheat and 35-63 days in corn (Fesenko, 1990).

2.4. Crop History

Early work on buckwheat improvement started in the CIS. The first developed variety with high nutritional quality and large grain size was 'Bogatyr' (Hero) released in the 1930s (Fesenko, 1990). Many tetraploid and determinate diploid cultivars have since been developed in the CIS. Tokyo, developed from two Japanese genotypes, was the first released

variety in Canada in 1955 (De Jong, 1972) with a small grain size. Attempts to develop autotetraploid varieties with a large seed size have had mediocre success as tetraploid species had much lower fertility than their diploid counterparts. Penquad, the first autotetraploid variety, was registered in USA in 1966 (Marshall, 1967). Although it was superior to Tokyo in the Pennsylvania environment, its yield potential in Canada was only 70 per cent of that of Tokyo (De Jong, 1972). Presently, large seeded cultivars such as Mancan and Manor have been developed from diploids (Campbell and Gubbels, 1986). Mutation breeding involving application of gamma rays and chemical mutagens has led to the development of large seeded cultivars with high grain yield and early maturity in the CIS (Alekseeva, 1988; Fesenko, 1990).

Although buckwheat has a heteromorphic, sporophytic self incompatibility system, a low degree of self fertility was reported as early as 1927 (Garber and Queensberry, 1927). Marshall (1969a) isolated a self fertile line, but it was very sensitive to temperature, expressing complete sterility above 25 C. Polycross and single cross hybrids based on inbred lines developed from such homomorphic lines expressed heterosis for seed yield in comparison to the inbred parents, but none of the hybrids outperformed the parental lines suggesting limited scope for hybrid breeding (Marshall, 1979). Selfing of homomorphic plants caused severe inbreeding

depression with yield reduced to approximately 40 per cent of parental line. Marshall (1970) was also able to develop and release an inbred line, 'Penline 10', for use in the development of homomorphic lines.

2.5 Flower Morphology of Buckwheat

Buckwheat flowers are borne in raceme at the ends of branches or on short pedicels arising from the leaf axil. Each flower consists of five parianths, a three parted style with knobbed stigmas, one superior ovary with a single ovule and eight stamens that are arranged in two whorls. The three inner stamens closely surround the style and protrude outward and the remaining five located in the outer whorl protrude inward. The flower has heteromorphic distyly. A flower with a long style and short stamens is called a pin flower (Fig 2.1), whereas a flower with long stamens and a short style is called a thrum flower (Fig 2.2). Each plant in a population bears either pin or thrum flowers.

At the time the flower opens, the stigma is fully receptive to pollen, but the anther dehisces sometime later (protogyny) which Krotov (1963) described as a mechanism of preventing self pollination in buckwheat. In contrast, Munshi (1989) reported that stigmas mature 15-17 hours after flower opening (protandry) in most cases in buckwheat flowers. Some

Fig. 2.1 Structure of a pin flower in buckwheat with a long style and short stamens.

Fig. 2.2 Structure of a thrum flower in buckwheat with a short style and long stamens.

authors have reported that plants adapted to bee pollination are protandry (Proctor and Yeo, 1973; Wyatt, 1983).

Genetically, incompatible reactions and morphological differences are controlled by alleles of a single gene. Sharma and Boyes (1961) postulated the involvement of a super gene, S, in buckwheat similar to that found in Primula. The short styled plants (thrum) are heterozygous (Ss) and the long styled plants (pin) are recessive homozygous (ss). Since compatible crosses are possible only between these two morphs, approximately a 1:1 proportion of both forms is present in a population. Although differences in the structure of the stigma have not been detected, a larger number of pollen grains with a smaller size is produced in pin flowers than those produced by thrum flowers (Doida, 1959; Krotov, 1963).

The sporophytic self-incompatibility reaction is controlled by the genotype of the pollen parent. Unlike homomorphic sporophytic system, the heteromorphic pollen can germinate on either form of the stigma, but the pollen tube growth is inhibited following pollination between the same form of flowers.

Morris (1947) observed pollen germination and pollen tube growth in vivo by making both compatible and incompatible pollinations. She noted that most of the pollen grains

germinated on the stigma irrespective of the compatibility and concluded that incompatibility was not associated with pollen germination. Cytological studies in legitimate pollination showed that the pollen tube reached the base of the style within 15 minutes of pollination. However, in illegitimate pollination, pollen germinated within 10 minutes and pollen tube growth was inhibited at the base of the stigma on thrum flowers, while it stopped after traversing two-thirds of the style on pin flower.

Ruszkowski (1981) studied the genetics of homomorphism, which he found to be controlled by two recessive genes. Self fertility was expressed only in double recessive homozygotes. Autogamous homomorphic lines have also been observed after colchicine treatment (Adachi, 1990). In such lines, inhibition of pollen tube growth at the stylar region was weaker than in normal line after illegitimate pollination.

2.6. Plant Breeding

A comparative study between tartary and common buckwheat revealed no significant differences with regards to plant vigor, dry and green matter accumulation, assimilation area, photosynthetic rate, root development and most yield components (Ruszkowski, 1981). The only difference was observed in the percentage seed set, suggesting self

incompatibility (SI) alone may be the cause of low fruit setting. Therefore, SI has been thought to be a major hindrance for yield improvement in buckwheat. One possibility for overcoming the problems with the SI system is to use F. tataricum, which is a self compatible species. Although conventional methods of combining F. tataricum genotype with F. esculentum have been unsuccessful (Morris, 1947), there is possibility of combining these species by somatic cell fusion.

Recently, anther culture and subsequent plant regeneration from callus have been reported (Bohanec, 1989). Development of haploids would facilitate morphological and genetical analysis in buckwheat, but unfortunately, all regenerants obtained so far are diploids (Adachi, 1990).

2.7. Crop Utilization

A field of buckwheat is a good source of nectar for honey production. The leaves are a rich source of rutin that has been used for the treatment of vascular disorders (Marshall and Pomerantz, 1982). Buckwheat flour, mixed with wheat flour, has been used to make noodle, pancake and bakery products. It has also been used in soup ingredients, mixed in breakfast cereals and health foods. The dehulled grains (groats) are cooked and served as rice, roasted and ground to

make tea or fermented to make liquor. The hulls are used for stuffing pillows in Japan and China. Due to the unique flavor of the flour, buckwheat preparations are considered delicacy in Japan. Buckwheat flour does not contain gluten as does wheat, but the presence of water soluble globulin and albumin in the aleurone layer produces enough stickiness for noodle making (Shiratori, 1986).

Buckwheat protein has a favorable composition of most of the essential amino acids, especially, lysine, threonine tryptophan and sulphur containing amino acids. Lysine, the first limiting amino acid in cereals at approximately at two per cent, is always over five per cent in buckwheat. Buckwheat protein has been considered one of the best sources of high biological value protein in the plant kingdom (Sure, 1955). Although the biological value of its protein is more than 90 per cent, its digestibility is less than 80 per cent owing to high crude fibre and tannin content (Javornik, 1983). Anti-nutritional factors such as trypsin inhibitors undermine the protein quality. The grain contains photo dynamically active dyes, which can produce a rash on white or light-colored areas of the skin or hide, under conditions of heavy consumption of buckwheat and exposure to sunlight, a condition known as 'Fagopyrism' (De Jong, 1972).

3. GERMINATION AND VIABILITY OF BUCKWHEAT POLLEN

3.1 Abstract

A large number of different media were tested in the development of an in vitro method for the germination of buckwheat pollen. Pollen grains were germinated successfully for the first time in an artificial medium consisting of 0.2 g each of $MnSO_4$, $Ca(NO_3)2.4H_2O$ and KNO_3, 0.04 g H_3BO_3, 15 g sucrose and 30 g polyethylene glycol (PEG) 20,000 dissolved in 100 ml of double distilled water. The pollen grains were considered germinated if they produced pollen tubes equal to or longer than the pollen grain diameter. The viability of the pollen was measured utilizing both in vivo and in vitro germination tests at temperature regimes of 20 and 25 C over a 38 hour period. Pollen grains were collected at four hour intervals from freshly harvested flowers grown under a 16 hour day length with a constant day and night temperature. The maximum pollen viability was obtained two hours and six hours after the first light when plants were maintained at 25 C and 20 C, respectively. Both tests showed that viability, as measured by germination percentage, was similar at both temperature regimes, except during the first six hours of the first light treatment. Although significantly lower germination was observed at 25 C than at 20 C on the following day, the differences were inconsistent. The pollen remained

viable for approximately 34 to 38 hours when collected from intact flower, but lost viability in less than an hour when stored at room temperature without humidity control. The viability was extended for more than an hour when high relative humidity was maintained at the same room temperature.

3.2 Introduction

A quick and reliable method of testing pollen viability is essential in studying environmental factors that affect pollen development. A pollen viability test would also be useful for breeders to identify male sterile lines, to assess the effect of pollen dispersed through insects and wind on pollination, and to determine the optimum time for making crosses to obtain maximum seed set.

The pollen viability in some species remains for less than an hour, but lasts for hours in others. Stephens and Quinby (1934) found that the longevity of sorghum (Sorghum bicolor L.) pollen was less than five hours when stored in the shade. Fritz and Lukaszewski (1989) reported that seed set in 'Chinese Spring' wheat (Triticum aestivum L.) decreased to less than 10 per cent after 45 minutes of pollen storage. The corn (Zea mays L.) pollen was viable for only three hours when stored in a pollinating bag in direct sunlight at 96 F, whereas it remained viable up to 30 hours when stored under a

shed at 86 F (Jones and Newell, 1948). Chiang (1974) reported that cabbage (Brassica oleracea variety capitata) pollen stored at room temperature showed a sharp reduction in germination within one day.

Tri-nucleate pollen grains are often difficult to grow in vitro and are short lived (Brewbaker and Quack, 1963; Shivanna and Johri, 1989). Buckwheat has a tri-nucleate pollen (Pandey, 1960) and may have a short life as well. It has been thought that temperature above 30 C is detrimental to pollen and flowers in buckwheat (Kreft, 1983). Thus, yield could be substantially reduced when the plants are subject to high temperatures during flowering. However, there is no method developed to assess the pollen viability and the effect of temperature on its life span is not known. The present study was, therefore, conducted to develop an in vitro germination technique for buckwheat pollen and to utilize this method to determine the effect of temperature and time on the longevity of pollen.

3.3 LITERATURE REVIEW

3.3.1 Factors Affecting Pollen Viability

The viability of pollen is considered to be greatly influenced by temperature, humidity, genotypic difference,

vigour and physiological stage of the plant and the age of the flower. The pertinent literature has been reviewed by Johri and Vasil (1961) and Shivanna and Johri (1989). In most species, low temperature and low relative humidity are favourable for pollen longevity except in the Gramineae, which requires low temperature and high relative humidity.

3.3.1.1 Temperature

The effect of high temperature on pollen viability has been reported in many crop species. Lapichino and Loy (1987) found an effect of high temperature not only on the germinability of mature pollen, but also on the subsequent germination of immature pollen grains in bottle gourd (Lagenaria siceraria). Plants exposed to a 7-hour treatment at 38 C failed to produce viable pollen for a day after the treatment. It took four days for them to recover to normal viability. Harrero and Johnson (1980) observed a genotypic response of corn pollen to temperature. In some genotypes, pollen germinated equally well at 27 and 32 C, but in others, germination was higher or lower at 32 C than at 27 C. All genotypes had a lower germination at 38 C than at 32 C. They concluded that prolonged exposure of plants to temperatures above 32 C reduced pollen germination of many corn genotypes.

3.3.1.2 Storage and Humidity

Wheat, sorghum, corn and triticale have been reported to have short-lived pollen. Stephens and Quinby (1934) examined the longevity of sorghum pollen by observing the quantity of seed produced in the ear after pollination. There was a reduction in seed set over time when pollinations were made every half hour using the same source of pollen collected 15 minutes after blooming. They found a decline in seed set from 60 to zero per cent in a five-hour period. Similarly, Fritz and Lukaszewski (1989) reported a very short period of viability for both wheat and triticale pollen. In their study, seed set in 'Chinese Spring' wheat was found to be 70 to 100 per cent after the first 15 to 20 minutes, but decreased to 30 to 50 per cent after 30 to 40 minutes. Less than 10 per cent germination was found after 45 minutes of storage. When the pollen grains were stored under normal green house conditions, some pollen grains survived up to 65 to 70 minutes in wheat and 110 to 120 minutes in triticale, but under desiccation, all pollen grains were found to be non-viable after 45 minutes and 75 to 80 minutes in wheat and triticale, respectively.

Similarly, the viability of corn pollen lasted for three hours when stored in a pollinating bag in direct sunlight at 96 F (Jones and Newell, 1948). However, they remained viable

for up to 30 hours when stored under a shed at 86 F suggesting that a cool temperature and high humidity extended the life of the corn pollen grains. Pope (1939) studied the longevity of barley (Hordeum vulgare L.) pollen at different storage conditions. He found that viability of barley pollen lasted for 26 days at 36 F, 19 days at 40 F and 14 days at 50 F.

Chiang (1974) examined the viability of cabbage pollen in storage. All pollen grains were found to lose their viability after a day's storage in a freezer. However, when stored at 4^0 C, germination was found to increase up to 10 days after storage and decreased thereafter. The higher germination found in pollen stored at 4^0 C as compared to the fresh pollen, suggested that a high moisture level can prevent germination. However, pollen stored at room temperature showed a sharp reduction in germination within one day, suggesting that a critical moisture level was not maintained at room temperature.

3.3.2 Tests for Pollen Viability

Viability of pollen was considered an indicator of the ability of the pollen grain to deliver the sperm cells to the embryo sac following compatible pollination (Shivanna et al, 1991). Barrow (1983), after examining different measures of pollen viability in cotton (Gossypium species), came to the

conclusion that the living pollen cells or germinable pollen grains tested by different staining and/or in vitro germination techniques were not always fertile. Since the main objective of measuring pollen viability was to assess the capacity of a pollen to fertilize the egg cell and induce the development of zygote, he recommended that examination of pollen tube penetration into the micropyle or the ovule and monitoring the percentage of seed set after pollination were the most reliable methods of estimating the pollen viability. However, these methods are tedious, time consuming and not always feasible (Heslop-Harrison et al, 1984). Therefore, several alternatives as indicators of pollen viability have been developed with different accuracy from species to species.

Each method has certain limitations and a single reliable method that is applicable to every species does not exist. For every method employed, it is important to define the conditions under which the pollen grains are collected and stored.

3.3.2.1 Tetrazolium Test

Vital stains such as 2,3,5-triphenyl tetrazolium chloride (TTC) are the most commonly used chemicals for testing pollen viability. The colourless TTC undergoes reduction by

dehydrogenase in living tissues and is converted into a coloured insoluble substance called formazan (Hauser and Morrison, 1964; Aslam et al, 1964). This gives a red or deep purple color in living cells. However, there is often a gradation of color from light red to dark red that makes it difficult to distinguish between dead and viable pollen (Shivanna and Johri, 1989). Aslam et al (1964) studied seven vital stains and found triphenyl tetrazolium chloride and tetrazolium red in 60 per cent sucrose solution were the best indicators for pollen viability in cotton. However, Oberle and Watson (1953) observed staining of dead pollen grains as well by TTC and concluded that it was a non reliable chemical to test the pollen viability in some fruit species such as peach, pear, apple and grapes.

Nevertheless, Rajora and Zsuffa (1986) found TTC to be a good indicator of pollen viability in poplar (Populous species) and confirmed this by in vitro germination and percentage of seed set tests in controlled crosses. Collins et al (1973) obtained similar results of pollen viability by staining with tetrazolium bromide and by an in vitro germination test in stored pollen of alfalfa (Medicago sativa L.), cotton (Gossypium species), soybean (Glycine max L.) and rye (Secale cereale L). The stored pollen required 50 to 100 per cent more time for staining and germination as compared to the fresh pollen. They found staining of pollen with

tetrazolium bromide superior to in vitro germination due to its simplicity in application. It also can be used for species where an artificial germination test is either not developed or unsatisfactory.

3.3.2.2 Nuclear Dyes

Staining of pollen with nuclear dyes, such as acetocarmine, iodine of potassium iodide, aniline blue in lactophenol, etc. were also considered as indicators of pollen viability in the past. The viable pollen grains have intact nuclear and cytoplasmic material that are stained by the dyes. Heslop-Harrison et al (1984) observed very low correlation of this test with germinability. In several studies, the acetocarmine stained dead as well as viable pollen grains alike and, thus, could not be regarded as an indicator of the pollen viability (Vasil, 1960).

3.3.2.3 Inorganic Acid Test

The viable pollen of some species rupture instantaneously when placed in an acidic medium. The cytoplasm coagulates when it is ejected from the cell and appears as a pollen tube. These tube like structures are stable and give a false impression of pollen germination unless they are checked for membrane integrity. A positive correlation between pollen

bursting and percentage seed set was obtained in cotton (Kearney and Harrison, 1932).

3.3.2.4 Fluorochromatic Test

Fluorochromatic test is based on the integrity of the plasmalemma and as it is likely that the integrity of the plasmalemma is closely correlated with viability, it was thought to provide an effective method of assessing pollen quality (Heslop-Harrison, 1970). Fluorescein diacetate (FDA) is a non-fluorescent, non-polar chemical that can freely enter the plasmalemma of living cells, where it is hydrolysed by the enzyme esterase to give a polar product, which cannot escape the intact membrane (Heslop-Harrison, 1970; Shivanna and Johri, 1989). As a result, it accumulates in the contents of the living vegetative cells and produces a bright fluorescence under fluorescence microscopy. Heslop-Harrison et al (1984) found a very high correlation between the FDA test and germinability, when mature pollen grains were used. Shivanna and Heslop-Harrison (1981) reported that the test gave a slight over estimate of the percentage of germinability. Heslop-Harrison et al (1984) concluded that the over estimation was possibly due to inclusion of immature pollen, which have intact membrane, but do not necessarily have all the enzymes required for germination. They further emphasized that FDA was not a test for viability, because germination,

considered as an indicator of pollen viability, occurs only at maturity and FDA being a test of membrane integrity, treats mature and immature pollen grains alike.

3.3.2.5 Seed Set Test

As the main function of a pollen grain is to deliver the male gametes into the embryo sac, the seed set test, based on the ability of pollen to fertilize the ovule and produce seed, was thought to give an accurate measure of pollen viability. Seed setting, however, does not depend only upon fertilization. Factors in the post pollination development of the ovule, environmental influence and physiological stress on the maternal plant can cause abortion of embryos or fruit that is beyond the function of the pollen (Barrow, 1983; Heslop-Harrison et al, 1984). Furthermore, this method is tedious, time consuming and difficult to put into practice.

3.3.2.6 In Vivo Germination Test

This may be the most reliable method for assessing pollen viability as one can test the germinability under natural conditions. It requires a technique to locate the pollen tubes in the pistil. The most satisfactory method presently used is fluorescent microscopy. The pollen tubes contain callose depositions that can readily be detected under

fluorescent microscopy upon staining with water soluble aniline blue (Martin, 1959; Jefferies and Belcher, 1974; Dumas and Knox, 1983). It is interesting that neither callose nor aniline blue is autofluorescent. But, when the two are in contact with the callose plugs, they fluoresce brightly in ultra violet (UV) light and can be clearly distinguished from the stylar tissue. Callose, which is generally composed of 1,3 B-glucans, also can be identified by staining with resorcinol blue (Eschrich and Currier, 1964). With either of the chemicals, pollen tubes can be readily detected in the pistil when viewed under a fluorescent microscope with a suitable filter.

3.3.2.7 In Vitro Germination Test

In vitro germination method is regarded as more reliable and accurate method than staining, under the assumption that a germinating pollen grain will be capable of fertilizing an egg cell and producing a functional zygote (Barrow, 1983). However, often pollen grains of some taxa do not germinate in artificial media and some others, although they germinate, fail to give satisfactory germination. Since the germination medium differs from species to species, finding a suitable medium for germination is a major problem that has yet to be solved in several economically important species. Furthermore, a medium that gives optimum germination of fresh

pollen may not be optimal for stored pollen. Shivanna and Johri (1989) reported that Antirrhinum pollen grains did not germinate in vitro, when stored beyond 180 days, however, after placing a piece of stigma in the medium, germination occurred beyond that period. In some species, stored pollen grains failed to germinate in vitro, but were capable of inducing satisfactory seed set (King, 1963).

3.3.3 Pollen Germination Medium

Several authors have reported on different requirements for in vitro germination of pollen. The osmotic potential of the medium, its pH and inclusion of various mineral salts and sucrose have been found to influence the germination of pollen in many species (Brewbaker and Kwack, 1963; Johri and vasil, 1961; Pfahler, 1968; Roberts et al, 1983). Pfahler (1968) reported that the medium for in vitro pollen germination depends upon the species. Distilled water or simple sucrose medium promoted excellent growth in some species, whereas the presence of additional substances was necessary in others. Difficulty in in vitro pollen germination of tri-nucleate pollen has been reported (Roberts et al, 1983; Johri and Shivanna, 1977). Although some species do germinate in vitro, the pollen tube length is short and does not relate to the distance they have to travel from stigma to the micropyle in order of fertilization to occur.

Wallace and Karbassi (1968) germinated oat (Avena sativa) pollen in a medium consisting of sucrose, raffinose, boric acid, calcium nitrate and bacto agar. They found bursting of pollen tubes and exudation of the cytoplasm within 6-8 minutes after seeding the pollen. They tried various measures such as changing the pH with different buffers, substitution of raffinose and sucrose as a source of sugar, agarose and gelatin instead of agar, varying temperature and relative humidity, altering concentration and substitution of minerals, addition of ground up stigmas, and enzymes and hormones to the medium etc. None of these alterations prevented bursting without inhibiting pollen germination.

Lee et al (1985) defined a medium consisting of 300 mg $CaCl_2.2H_2O$, 100 mg KNO_3, and 10 mg of H_3BO_3 dissolved in one litre of water along with 20 per cent sucrose and 4 to 5 per cent agar for germination of jojoba (Simmondsia chinensis) pollen. They found some germination in a wide range of medium constituents and pH. The most important factors influencing germination were sucrose and agar concentration, temperature and relative humidity. The germinating plates with the covers open produced only 15 per cent germination as compared to 65 per cent when the plates were covered with lids indicating that relative humidity was a critical factor in pollen germination.

Ferrari and wallace (1975) found that in vitro germination of cabbage pollen was dependent upon the addition of purified polyethylene glycol (PEG) 20,000 as a supplement to the nutrient medium containing sucrose. The optimum concentration of PEG varied from 15 per cent to 30 per cent depending upon the genotype.

3.3.3.1 Effect of Calcium and Boron

Calcium has been found to be associated with binding in the cell wall and thus, increases rigidity and stability of the cell wall. Boron is incorporated into sugar-borate complexes that allows increased absorption, translocation and metabolism of sugar and increases oxygen absorption and synthesis of pectin material for the newly formed pollen tube walls (Linskens, 1964; Pfahler, 1967). Pfahler (1967) observed increases in germination and pollen tube elongation of corn pollen with the addition of calcium nitrate and boric acid in a sucrose-agar medium. He later found a strong influence of calcium, boron and pollen source on the elongation of the pollen tubes (Pfahler, 1968). For all genotypes tested, the rate of pollen tubes growth and their final length was greater in a medium with a combination of calcium and boron than in those with other compounds.

Ferrari and Wallace (1975) found acceleration of cabbage

pollen germination with the addition of boric acid and calcium chloride. However, at high concentration, they had an inhibitory effect on pollen germination. Pollen tube elongation was found to be less sensitive than pollen germination at low level of these compounds. Chiang (1974) found calcium and boron not essential for the germination of cabbage pollen, but highly beneficial when added at the optimum levels. Application of boron increased germination as well as pollen tube elongation in Eucalyptus pollen until its concentration was raised to 100 ppm (Potts and Smedley, 1989).

3.3.3.2 Effect of Sucrose

There was no difference in germination of alfalfa pollen at sucrose concentrations of 12 to 24 per cent (Lehman and Puri, 1964). However, pollen tube growth was found to be poorer at lower levels of sucrose. It improved progressively as the sucrose concentration increased, with the longest tubes being produced at 20 per cent. Ferrari and Wallace (1975) reported the optimum level of sucrose concentration as 0.6 M for germination as well as pollen tube elongation in cabbage pollen. Sucrose concentration above 40 per cent impaired pollen germination as well as pollen tube growth, whereas a concentration below two per cent led to bursting of pollen in most plant species observed (Brewbaker and Kwack, 1963).

Chiang (1974) tested 16 different sugars and found only six of them (sucrose, raffinose, lactose, maltose, melizitose and trehalose) supported germination of cabbage pollen. In her studies, sucrose produced the highest pollen germination, whereas raffinose produced the longest pollen tubes. Rajora and Zsuffa (1986) found sucrose concentration in a range of 10 to 20 per cent as optimum for in vitro germination of poplar pollen, depending upon the species. Potts and Smedley (1989) found an effect of sucrose on pollen germination as well as tube elongation on Eucalyptus pollen. Optimum germination was obtained at a 30 per cent sucrose concentration, whereas the tube length was maximum at 20 per cent sucrose. Very little germination was found without sucrose.

3.3.3.3 Effect of Pollen Density

The optimum pollen concentration in cabbage was found between 0.5 to 20 mg pollen per ml of germination medium (Ferrari and Wallace, 1975). With the increase in number of anthers (as a pollen source) from 1.5 per 0.05 ml medium, there was proportionately lower germination and shorter pollen tube growth. No germination was found when the anther number was increased to four indicating high pollen concentration inhibited pollen germination.

In contrast, Roberts et al (1983) found an increase in

cabbage pollen germination from 60 to 90 per cent by increasing the pollen concentration from two to 50 g per mm^2 in the medium. Likewise, a small population of pollen rarely germinated in vitro, but the germination as well as tube elongation proportionately increased with the increase in pollen density (Brewbaker and Kwack, 1963). They found a population effect on pollen germination as well as pollen tube elongation on all 86 flowering plants covering 79 genera and 39 families. The population effect was overcome by the addition of calcium in medium in all the species they examined.

3.3.3.4 Effect of pH

Ferrari and Wallace (1975) examined the effect of pH on cabbage pollen germination and pollen tube growth. The highest germination was obtained at a pH of 5.8 with no germination being found at a pH of 7.0. There was a slight increase in germination with an increase in pH from 4.3 to 5.8, but pollen tube elongation was almost doubled. Roberts et al (1983) found a dramatic increase in germination of cabbage pollen, from 20 to 80 per cent, when the pH was increased from 6 to the range of 8-9. Tris buffer was found to be more effective in stimulating pollen germination than other buffers. Hepes buffer gave 20 per cent germination but phosphate buffer gave no germination. A medium adjusted with

NaOH produced far less pollen tubes than that of Tris at the same pH level. However, Lehman and Puri (1964) found no difference in alfalfa pollen germination in a range of pH from 5.5 to 8.0.

3.3.3.5 Effect of Other Substances

Brewbaker and Kwack (1963) tested organic substances such as coconut milk, yeast extract, plant growth regulators and different amino acids alone or in various combinations in germination media. They found no effect of these substances in enhancing pollen germination except coconut milk and yeast extract. Similarly, Ferrari and Wallace (1975) did not find any beneficial effect from the addition of mannitol, amino acids, glycerol, paraffin, yeast extract, clove oil and KCl on cabbage pollen germination. Elevated level of atmospheric CO_2 was found to double cabbage pollen germination in the absence of Tris (Roberts et al, 1983). However, there was no such effect in the presence of Tris. Reduction in CO_2 concentration caused a decrease in germination under both conditions. The addition of K, Na and Mg served a supporting role for the uptake or binding of Ca (Brewbaker and Kwack, 1963).

3.4 MATERIALS AND METHODS

3.4.1 Pollen Germination Medium

A large number of different media reported to germinate pollen of other crop species were prepared and tested (Brewbaker and Kwack, 1963; Wallace and Karbassi, 1968; Taylor, 1972; Pfahler, 1967 and 1968; Chiang, 1974; Barrow, 1981; Sahar & Spiegel-Roy, 1984; Lee et al, 1985; Rajora and Zsuffa, 1986; Burns, 1989). Most of these media consisted of salts of Ca, Mg or Mn and K, B and sucrose with or without agar. Then, media with 27 (3x3x3) combinations of $MnSO_4$, $Ca(NO_3)_2.4H_2O$ and KNO_3 at the rate of 0.0375, 0.075 and 0.15 g were prepared keeping the constant level of H_3BO_3 (0.04 g) and sucrose (30 g) dissolved in 100 ml of double distilled water. In further experiment, media with eight (2x2x2) combinations with higher level of these mineral salts (0.3 and 0.45 g) were prepared. The next experiment consisted of another eight combinations of 0.2 and 0.25 g of the mineral salts. In the next step, a medium comprising 0.2 g each of these salts was prepared and designated as 'basic medium', which was then modified with different levels of sucrose (30, 40 and 50 per cent), H_3BO_3 (0.01, 0.04 and 0.08 per cent) and pH (5-8).

In the final experiment, the basic medium added with 0.04 g H_3BO_3 was altered using four levels of sucrose (10, 15, 20

and 25 per cent) and four levels of PEG 20,000 (20, 25, 30 and 35 per cent). The level of PEG and sucrose was optimized by keeping one of the variables constant and adjusting the other. After adjusting the PEG and sucrose concentration, the medium was described as 'standard medium'. The pH of the standard medium was then modified to range from 5.0 to 8.0 by adding NaOH or HCl. The pH was adjusted after all the ingredients of the medium except PEG had been dissolved.

After defining the standard medium, a stock solution consisting 0.2 g each of $MnSO_4$, $Ca(NO_3)2.4H_2O$ and KNO_3, and 0.04 g H_3BO_3 was prepared by dissolving each salt in 100 ml double distilled water. Each day, a fresh standard medium was prepared by dissolving 1.5 g sucrose and 3 g PEG in 10 ml of the stock solution for the pollen viability studies.

Pollen grains were collected from freshly harvested flowers grown in a green house under a 16 hour day length and at 20/15 C day/night temperature. They were germinated on microscope slides in the standard medium described above. A drop of the medium was placed on the slide and anthers from a single flower were dipped gently in the medium and immediately removed. The slide was kept in a petri dish containing a wet paper towel and covered with a lid to maintain a high relative humidity. Although preliminary examination showed germination of the pollen grain in 10 to 15 minutes, the slides were

incubated at room temperature for 40 minutes in order to ensure adequate time for maximum germination. After incubation, the pollen grains were examined under a Zeiss compound microscope at 10X and rated as germinated, not germinated or burst. Pollen grains were considered germinated if the pollen tubes were equal to or longer than the pollen diameter (Fig. 3.1.A and B). Bursting of the pollen grains was identified by an irregular mass of cytoplasm and starch grains protruding from the pollen wall (Fig.3.2.A and B).

3.4.2 Pollen Viability Test

After the development of a pollen germination medium for buckwheat, pollen viability studies were conducted utilizing in vivo and in vitro germination tests at two temperature regimes, collecting pollen from a single cultivar, Manor. The plants were maintained at a constant day and night temperature of 20 C in one growth cabinet and 25 C in another with a 16 hour day length throughout the study period. They were placed in the cabinet five days prior to taking the first observation to acclimatize the plants to the growing conditions. The daily temperature inside the cabinet was ensured by monitoring it with a thermometer placed beside the plants. All opened flowers were removed from the plant one day before the commencement of the experiment. Each morning, one hour after light, freshly opened thrum flowers were randomly chosen and

marked with a felt marker. Flowers opened on the first day of observation were marked with red and those opened on the second day were marked with black, with the sequence being followed until the end of the experiment. Only marked flowers were used as a source of pollen to ensure uniform age of the flowers.

Although a preliminary study showed no difference in pollen germination percentage between pin and thrum flowers, only thrum flowers were used. Their long stamens facilitated seeding of pollen in the medium and increased the ease of pollination of the pin flowers while conducting the in vivo germination test.

3.4.2.1 In Vivo Germination Test

The styles in the pin flowers of buckwheat are longer than the stamens that help prevent self pollination in such flowers. Therefore, the pin flowers were used as a source of stigmas to avoid the tedious and time consuming emasculation process. The pin and thrum flowers were harvested separately from plants grown in growth cabinets as described above. Stigmas along with the style were excised from the pin flowers and were examined under a stereo microscope to ensure their freedom from any pollen. They were then pollinated using pollen grains from the thrum flowers, that were harvested

simultaneously. Each style was then placed separately on a microscope slide and kept in a petri dish with a moist paper towel at either 20 or 25 C to allow for pollen germination.

After 40 minutes of incubation, the styles were immersed in a drop of 0.05 per cent water soluble aniline blue prepared by dissolving it in 0.1 M K_2PO_4 having pH 7.2 for 2-3 minutes and were then covered with a cover slip. The slide was observed under a Nikon epifluorescent microscope magnified at 10x20 in a darkened room. The pollen tubes fluoresced a bright yellow-green due to the presence of callose and contrasted strongly with the stylar tissue. At least 50 pollen grains attached to the stigmatic surface were observed on the six stigma lobes from two styles for each observation. Several pollen tubes were found to have penetrated the stigmatic surface (Fig.3.3). Pollen grains producing pollen tubes longer than or equal to the pollen diameter (regardless of penetration of the stigmatic surface) were regarded as viable with the rest being rated as nonviable.

3.4.2.2 In Vitro Germination Test

Pollen grains were germinated on the microscope slide in the standard medium as previously described (3.4.1). The petri plates containing pollen grains were incubated at the same temperature, of either 20 or 25 C, as that under which

the plants had been maintained. After 40 minutes of incubation, a minimum of 50 pollen grains were counted under a Zeiss compound microscope magnified at 10x10 for each sample and rated as being germinated or non-germinated. Pollen grains were considered germinated if the pollen tubes were equal to or longer than the pollen diameter. A field containing the pollen from a single flower was regarded as a sample and each observation consisted of four such fields. The mean germination of these samples was recorded as the percentage of pollen viability for each four hour intervals.

The same procedure was followed for both methods with the exception of the germination medium. The pollination and viability assessment was done at four-hour intervals beginning at two hours after the first light and continuing for 34 to 38 hours until the germination decreased to less than five per cent. This procedure was repeated four times with each repeat being considered as one replicate in each of the two temperature regimes. Due to a fairly wide range of germination percentage over time, the data were arcsine transformed prior to statistical analysis. Analysis of variance was performed using a randomized complete block design to determine the effect of temperature and flower age on pollen viability. The germination percentage at each observation period was compared between the two temperature regimes using the paired T-Test for both methods.

3.5 RESULTS AND DISCUSSION

3.5.1 Pollen Germination Medium

This is the first report of a medium for the in vitro germination of buckwheat pollen. The medium consisted of 0.2 g of each of $MnSO_4$, $Ca(NO_3)2.4H_2O$ and KNO_3, 0.04 g H_3BO_3, 15 g sucrose and 30 g PEG 20,000 all dissolved in 100 ml of double distilled water. Pollen grains were found to germinate within 10 to 15 minutes after seeding. Up to 84 per cent of the pollen grains were found to germinate in the medium. Pollen tubes were observed to be several times longer than the diameter of pollen grain in most cases (Fig 3.1.A). Some pollen grains were found to produce double (Fig 3.1.C) and occasionally triple pollen tubes approximately the same length. Occasional branching of the pollen tubes was also observed.

Before successful development of the medium, a large number of media described to germinate pollen in other crop species were tested (Brewbaker and Kwack, 1963; Wallace and Karbassi, 1968; Taylor, 1972; Pfahler, 1967 & 1968; Chiang, 1974; Barrow, 1981; Sahar & Spiegel-Roy, 1984; Lee et al, 1985; Rajora and Zsuffa, 1986; Burns, 1989). Buckwheat pollen grains did not germinate in any of these media. Media with various combinations and concentration of salts of Ca, Mg or

Mn and K, boric acid and sucrose with or without agar were, then, prepared and tested for pollen germination. Pollen grain swelled and burst, without any germination in each medium with all 27 (3x3x3) combinations of $MnSO_4$, $Ca(NO_3)_2.4H_2O$ and KNO_3 at the rate of 0.0375, 0.075 and 0.15 per cent with a constant level of H_3BO_3 (0.04 per cent) and sucrose (30 per cent). Inclusion of two per cent Bacto agar in this medium did not stop bursting (Fig. 3.2.B).

The ruptured pollen ejected cytoplasm that coagulated in the nutrient medium. The exuded mass was fairly stable and resembled pollen tube growth (Fig. 3.2). Bursting of pollen was confirmed by the fluorescence of callose after excitation with UV light in the presence of aniline blue. The ruptured pollen tube like structures failed to give fluorescence as they lacked intact cell wall containing callose, a fluorescent substance. The percentage of pollen bursting was found to be correlated with pollen viability as determined by in vivo and in vitro germination tests (Fig.3.9). Similar results have been reported by Kearney and Harrison (1932) in cotton.

When the combined salt concentration was increased to 0.3 per cent, two to three per cent germination was found that encouraged further work on germination medium. However, subsequent increase in mineral concentration was found to inhibit all germination.

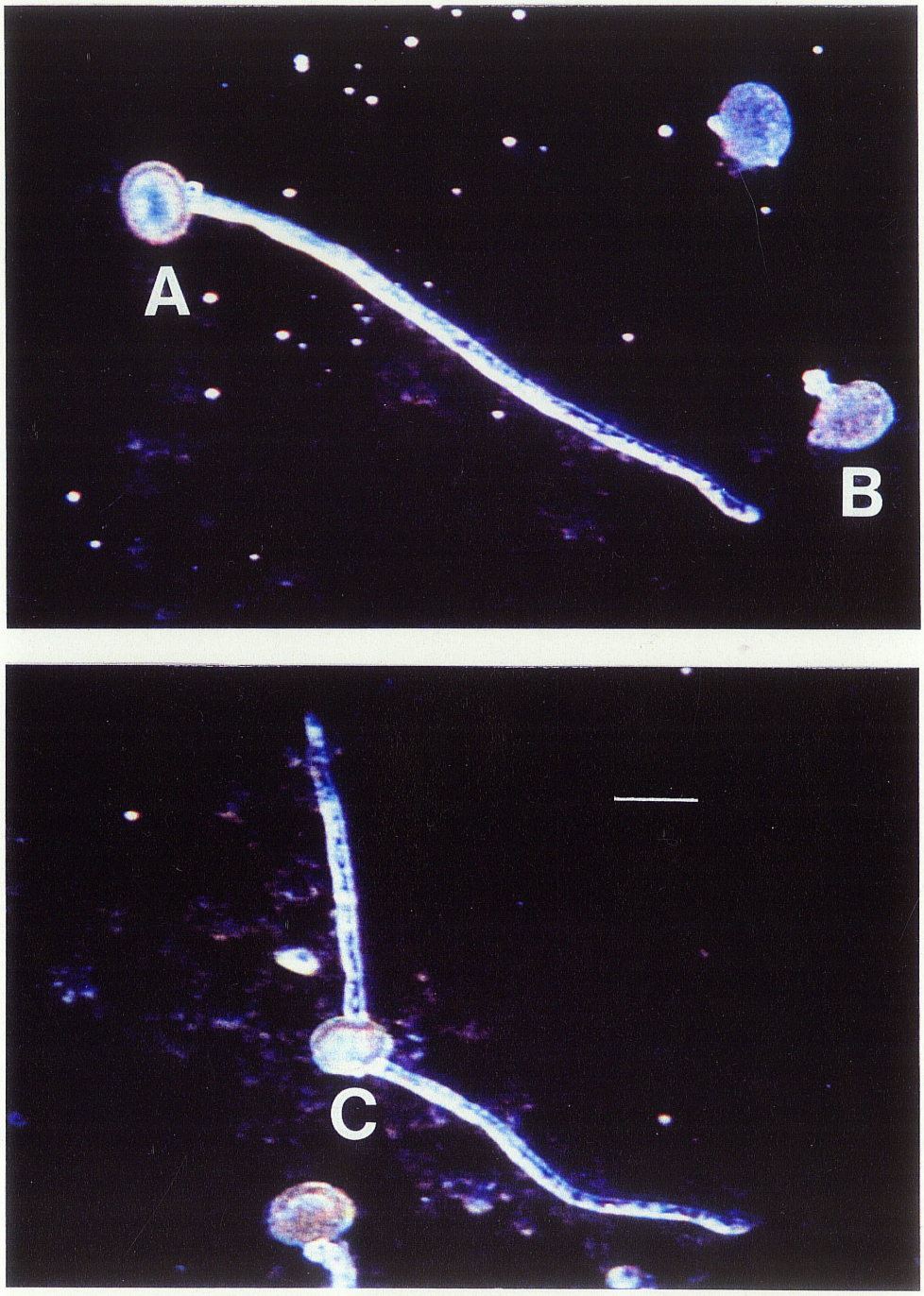

Fig. 3.1 Germination of buckwheat pollen in the standard medium: (A.) Normal pollen germination, (B.) Budding of pollen, not considered germinated, (C.) Double pollen tubes arising from the same pollen grain. (Scale bar represents 50 um.)

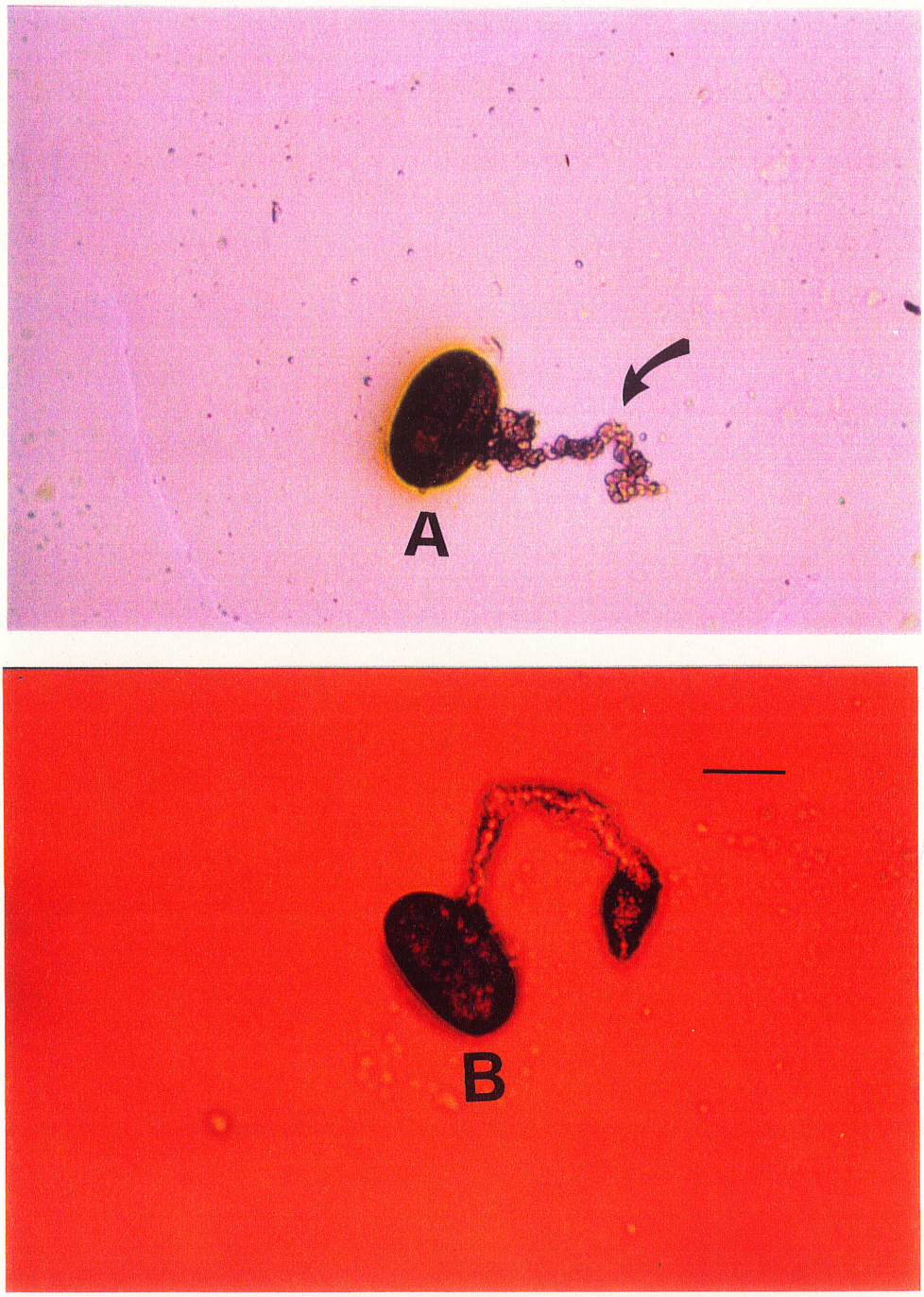

Fig. 3.2 Buckwheat pollen showing bursting: (A.) In a medium containing 0.075 % each of $MnSO_4$ and KNO_3, 0.15 % $Ca(NO_3)_2.4H_2O$, 0.04 % H_3BO_3 and 30 % sucrose; (B.) in the same medium supplemented with 2 % bacto-agar. Arrow shows the exudation of starch and cytoplasm. (Scale bar represents 25 um.)

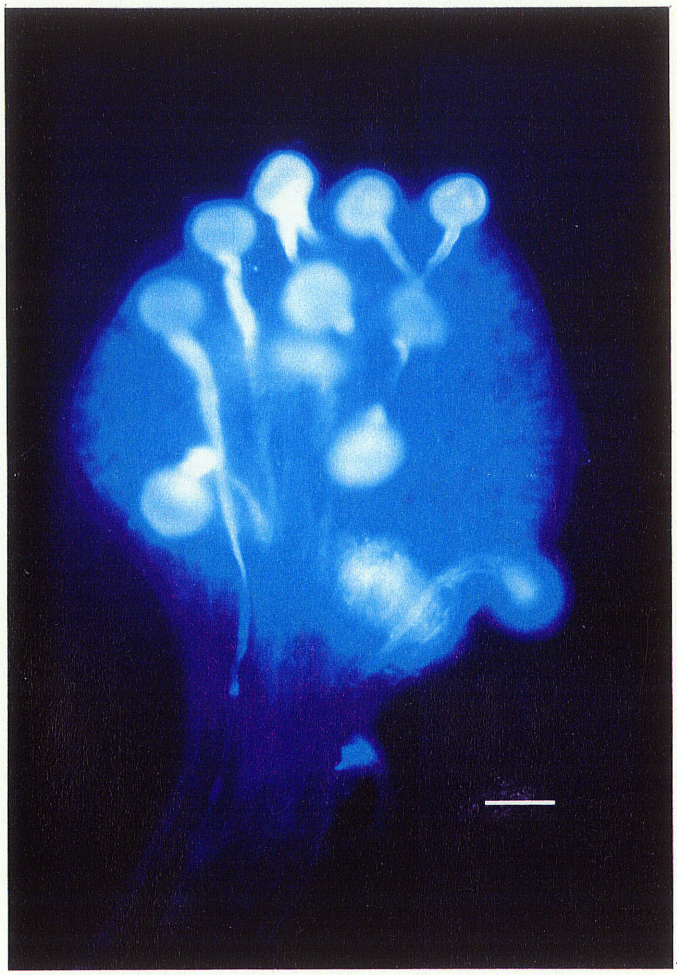

Fig. 3.3 Germination of buckwheat pollen on the stigmatic surface. (Scale bar represents 50 um.)

There was approximately 13 per cent pollen germination in the basic medium supplemented with 0.04 per cent H_3BO_3 and 30 per cent sucrose. Pollen tubes having smooth walls were observed that were several times longer than the pollen grain diameter. Higher level of any individual salt was found to inhibit germination. Substitution of $MnSO_4$ with $MgSO_4$ failed to produce any germination. Likewise, exclusion of anyone salt among the three was found to inhibit germination. Therefore, all the three salts were considered as essential for buckwheat pollen germination and further research was continued with these salts.

An increase or decrease in the level of H_3BO_3 in the basic medium was found to inhibit pollen germination (Table 3.1). However, when the level of sucrose was increased to 40 per cent, germination was improved from 13 to 24 per cent (Table 3.2). Increase in sucrose concentration to 50 per cent was found to produce neither germination nor bursting of pollen.

Table 3.1 Germination of buckwheat pollen in a medium containing 0.2 % each of $MnSO_4$, $Ca(NO_3)_2.4H_2O$ and KNO_3, and 30 % sucrose as affected by H_3BO_3 concentration.

% H_3BO_3	% Germination
0.01	04.0 ± 2.27
0.04	12.7 ± 1.30
0.08	approx. 1.0

Table 3.2 Germination of buckwheat pollen in a medium containing 0.2 % each of $MnSO_4$, $Ca(NO_3)_2.4H_2O$ and KNO_3, and 0.04 % H_3BO_3 as affected by sucrose concentration.

% Sucrose	% Germination
30	13.0 ± 5.6
40	24.3 ± 4.9
50	0

Other attempts to enhance pollen germination and stop bursting were modification of pH with and without buffer, aging the medium, desiccation or freezing of pollen grains, addition of ground up stigmas or inclusion of different levels of agar in the medium. Such endeavours neither increased germination nor stopped rupturing of pollen grains. Similar results were obtained by Wallace and Karbassi (1968) in oats.

The addition of 30 per cent PEG 20,000 and 15 per cent sucrose to the basic medium with 0.04 per cent H_3BO_3, however, increased germination almost four fold. Pollen grains were found to have germinated within 10 to 15 minutes of seeding in the medium. The pollen tubes observed were quite long with some as long as 620 um. The tubes were stable and did not detach even after squashing with the cover slip. Substitution of PEG 20,000 with a lower molecular weight (3,500 and 5,000) was found to produce bursting of the pollen. The ejected

cytoplasm produced long, smooth and stable pollen tube like structure that resembled pollen tubes. These tube like structures were different from those produced without PEG in that the former had smooth membrane like structure surrounding the dense cytoplasm, whereas the latter had discontinuous, spiral like structure covering the thin cytoplasm.

Some pollen grains were found to have double pollen tubes. Pfahler (1968) found germination of two pollen tubes from a single corn pollen grain, which he considered to be characteristics of various corn hybrids. Expression was found to be influenced by the presence of calcium and boron. In this study, no double tubes were observed in the absence of PEG indicating possible influence of PEG on producing double tubes from a single pollen grain.

No germination was obtained without the addition of sucrose to the medium. Approximately 50 per cent germination was obtained at 10 per cent sucrose level (Fig. 3.4). The germination percentage was found to increase with an increase in sucrose concentration. Approximately, 35 per cent of the pollen grains were found to have burst at 10 per cent sucrose level, but it was found to be negligible at 15 per cent producing up to 84 per cent pollen germination. Increasing the sucrose concentration from 15 to 25 per cent decreased germination as well as pollen tube length, but increased

bursting. The longest pollen tube at 25 per cent sucrose was found to be approximately 1.5 times the diameter of the pollen grain, while the majority of the tubes were equal to the length of the pollen diameter. Most of the tubes were found to be ruptured at the distal end. No double pollen tubes were observed possibly due to bursting of the tubes before elongation.

Similarly, there was also an effect of PEG concentration on germination, tube elongation and bursting of pollen grains. Pollen grains failed to germinate at the 10 per cent level of PEG with 15 per cent sucrose. They were found to germinate at 20 per cent PEG level with germination increasing with increasing PEG concentration (Fig 3.5). Maximum germination was obtained at 30 per cent PEG concentration. At this level, there was very little bursting of pollen and distal ends of the tubes were smooth with an intact wall. An increase in PEG concentration from 20 to 25 per cent was found to have no marked effect on pollen germination. There were, however, major differences in pollen grain bursting. When the level was increased beyond 30 per cent, there was decrease in pollen germination, but an increase in pollen bursting. Pollen tubes were found to be short which burst at the distal end. Upon bursting, a thick mass of cytoplasm accumulated at the tip. The ruptured pollen grains were also observed to exude cytoplasm from their walls at several points.

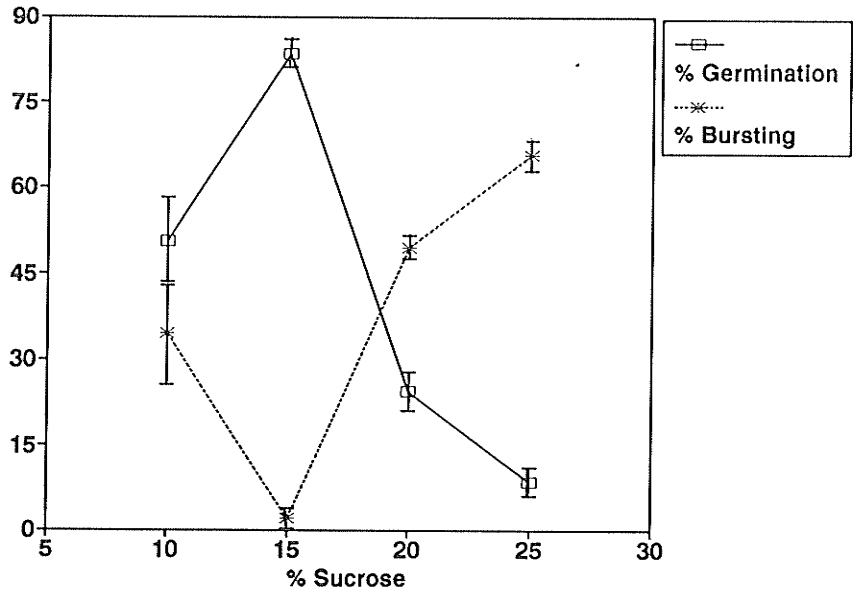

Fig. 3.4 Germination of buckwheat pollen as affected by concentration of sucrose in the basic medium supplemented with 30 % PEG 20,000 and 0.04 % boric acid. Vertical bar represents standard deviation.

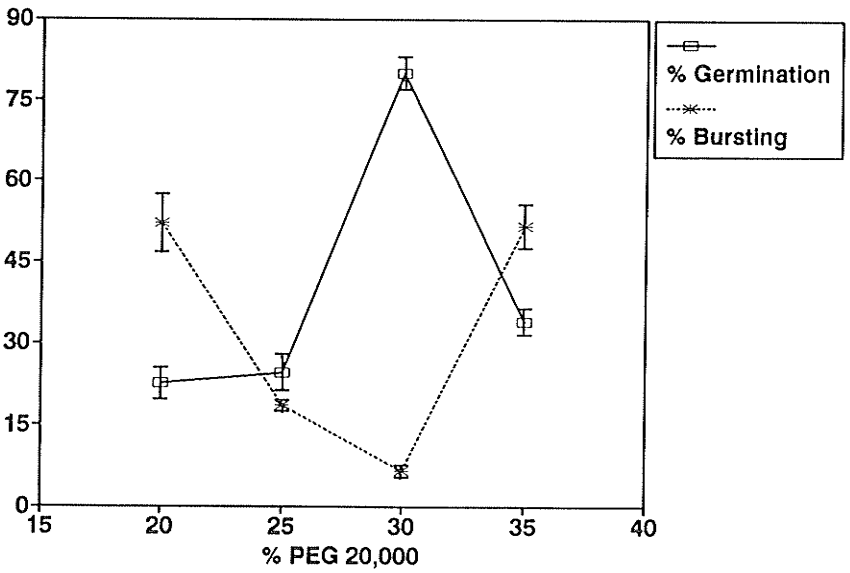

Fig. 3.5 Germination of buckwheat pollen as affected by concentration of PEG 20,000 in the basic medium supplemented with 15 % sucrose and 0.04 % boric acid. Vertical bar represents standard deviation.

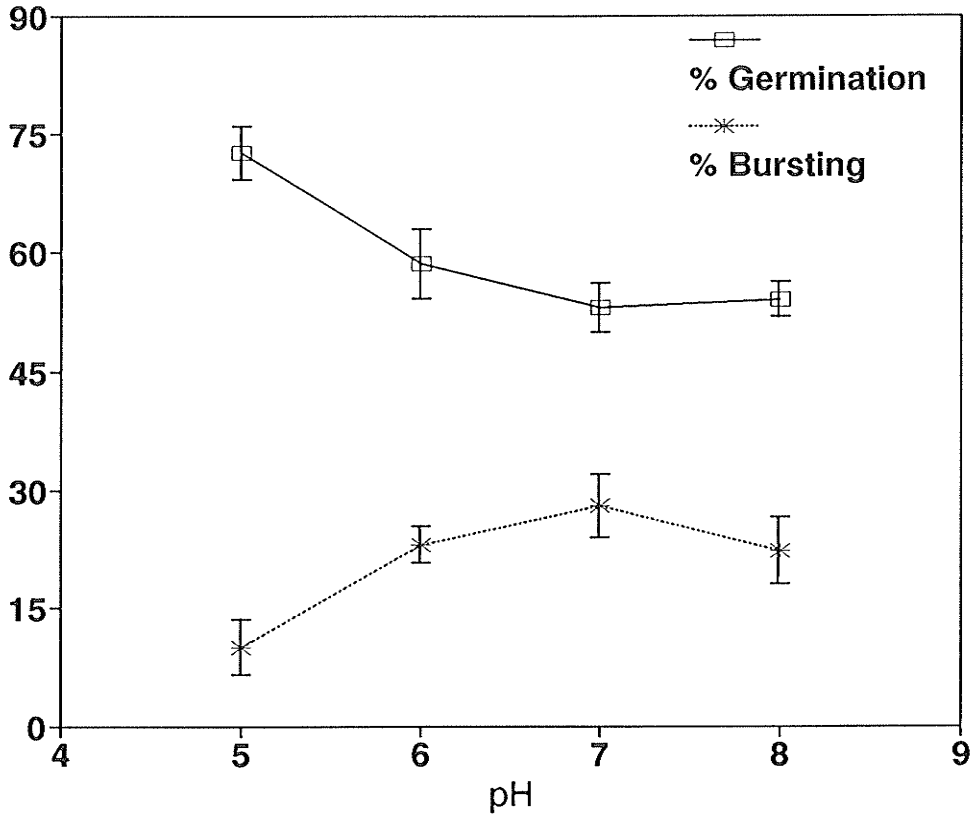

Fig. 3.6 Germination of buckwheat pollen in the standard medium as affected by levels of pH. Vertical bar represents standard deviation.

It has been reported that absorption of PEG by plant cell was inversely related to its molecular size (Janes, 1974). This indicates that high molecular weight PEG was possibly not absorbed by pollen, but worked as an osmoticum in the medium. Theoretically, when the osmotic pressure of a medium is lower than that of the pollen, water is forced into the pollen grain from the medium. As a result, the pollen wall cannot sustain the increase in internal pressure and the pollen grain bursts. However, in the present study, bursting of pollen was observed at low as well as high level of PEG and sucrose. The mechanism causing the bursting of pollen was not determined in this study.

Pollen grains did not germinate unless a moist paper towel was placed in the petri plate containing pollen slide, indicating high relative humidity was required for germination. Similar results have been reported by Lee et al (1985) in the germination of jojoba pollen. In their studies, they obtained only 15 per cent pollen germination when the petri plate containing the pollen slide was left uncovered. When the petri plate was covered with a lid, the germination increased to 65 per cent.

Maximum germination of pollen was observed at a pH 5.0, but decreased at pH 6.0. However, pH had no influence on germination ranging from 6-8 (Fig 3.6). It has been reported

that the pH of the stigmas before pollination was approximately 5 in buckwheat flower, corresponding to the pH in the present medium (Krotov, 1963).

3.5.2 Pollen Viability Test

Buckwheat pollen grains were found to have entirely lost their viability within one hour after collection when they were stored at room temperature (23 C ± 1) unless a high relative humidity was maintained. Some pollen grains retained viability for approximately three hours when they were stored in a petri dish, with a moist paper towel at the same room temperature (Table 3.3). This suggests that high relative humidity not only is required for pollen germination, but also for retaining the longevity of the pollen.

Table 3.3 Germination Percentage of Buckwheat Pollen Over Time Stored at Room Temperature (23 ±1) Maintaining a High Relative Humidity.

Hours	% Germination
0	75.30 ± 9.44
1	66.67 ± 9.76
2	16.23 ± 8.15
3	02.12 ± 1.23
4	00.00

3.5.2.1 In Vivo Germination Test

Pollen viability, as measured by germination percentage, was found to be dependent upon the temperature regime only during the first few hours after flower opening (Fig 3.7). At 20 C, the maximum viability observed at six hours after the first light, was significantly higher than at any other time (Table 3.4). The viability remained constant between the 10 and 14 hour observations, and did not differ statistically from that recorded at the two hour observation. A sharp reduction in germination occurred when the light was turned off at 18 hour. Viability thereafter decreased gradually reaching approximately 5 per cent after 34 hour.

Maximum pollen germination (78 %) was observed at two hour after the start of the first light when the plants were maintained at 25 C, unlike that observed at 20 C (Fig 3.7). Germination percentage decreased thereafter but remained constant from the 6 to the 14 hour observation (Table 3.5). The decrease in germination was greater at 25 C than at 20 C when the light was turned off at 18 hours. The overall germination percentage decreased in approximately the same manner as that observed at 20 C. Under both temperature regimes, the main effect of temperature was noted within the first six hours after first light. Although a significant effect of temperature was observed at 22 hour after the first

light, the effect was insignificant at other observation times on the following day (Table 3.8).

Table 3.4 Duncan's Means Separation Test Results for Buckwheat Pollen Germination at Four-Hour Intervals as Measured by In Vivo Germination Method (Under 20 C).

Hours After the First Light	Mean Germination (Data Arcsine Transformed)
2	0.920 b*
6	1.126 a
10	0.926 b
14	0.933 b
18	0.668 c
22	0.557 cd
26	0.451 de
30	0.327 ef
34	0.221 f

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 3.5 Duncan's Multiple Means Separation Test Results for Buckwheat Pollen Germination at Four-Hour Intervals as Measured by In Vivo Germination Method (Under 25 C).

Hours After the First Light	Mean Germination (Data Arcsine Transformed)
2	1.084 a*
6	0.883 b
10	0.858 b
14	0.906 b
18	0.509 c
22	0.464 c
26	0.365 c
30	0.210 d
34	0.128 d

* Means followed by the same letter are not significantly different at 0.05 probability level.

3.5.2.2 In Vitro Germination Test

Maximum viability was found six hours after first light and remained constant for next four hours when the plants were maintained at 20 C (Fig.3.8). It decreased significantly after 14 hours and remained constant for rest of the light and dark period thereafter (Table 3.6). However, the germination was found to decrease gradually during next light period.

Table 3.6 Duncan's Means Separation Test Results for Buckwheat Pollen Germination at Four-Hour Intervals as Measured by In Vitro Germination Method (Under 20 C).

Hours After the First Light	Mean Germination (Data Arcsine Transformed)
2	0.804 b*
6	1.006 a
10	0.950 a
14	0.796 b
18	0.798 b
22	0.739 bc
26	0.657 c
30	0.498 d
34	0.319 e
38	0.253 e

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 3.7 Duncan's Means Separation Test Results for Buckwheat Pollen Germination at Four-Hour Intervals as Measured by In Vitro Germination Method (Under 25 C).

Hours After the First Light	Mean Germination (Data Arcsine Transformed)
2	1.002 a*
6	0.995 a
10	0.920 a
14	0.769 bc
18	0.815 b
22	0.704 c
26	0.561 d
30	0.411 e
34	0.266 f
38	0.146 g

* Means followed by the same letter are not significantly different at 0.05 probability level.

At 25 C, maximum viability occurred two hours after initiation of the light period. Unlike in vivo germination test, the viability did not decrease rapidly, but remained stable for the next eight hours. It was found to decrease significantly at 14 h and remained almost constant for the rest of the light and dark period (Table 3.7) as observed in the similar test at 20 C. After 6 hours of light, the decrease in germination percentage was similar to that

observed at 20 C. Unlike the in vivo test, the change from light to dark did not decrease germination percentage. After 18 hour, the germination percentage decreased significantly at each observation period. The overall viability was retained for 38 hours.

Table 3.8 Paired T-Test Results Comparing Pollen Germination at Each Observation Period Under 20 C and 25 C as Measured by In Vivo and In Vitro Germination Methods.

Hours After The First Light	DF	Prob > |T|	
		In Vivo Method	In Vitro Method
2	7	0.0002	0.0070
6	7	0.0061	0.5856
10	7	0.1826	0.6297
14	7	0.5468	0.6868
18	7	0.0185	0.6259
22	7	0.5937	0.4355
26	7	0.0045	0.0495
30	7	0.1750	0.0487
34	7	0.2782	0.0274
38	7	-	0.1271

Germination of pollen was affected by temperature mainly during the first 6 hours after the first light (Table 3.8). Although a significant effect of temperature on germination

was observed from 26 to 34 hours when tested by in vitro method, the effect was not consistent by in vivo method.

The maximum pollen germination found by in vivo as well as in vitro germination method was approximately 84 per cent. The complete loss of pollen viability in less than an hour indicates that buckwheat pollen is very delicate. It, therefore, seems possible that some of the pollen grains could have lost viability between flower harvesting and their utilization in viability tests. A short period of pollen viability has also been reported by Fritz and Lukaszewski (1989) where they found a complete loss of viability within 65 to 70 minutes in wheat and 110 to 120 minutes in triticale under green house conditions.

The level of relative humidity in both the growth cabinets was found to be high while conducting in vitro germination tests at both temperature regimes. Therefore, a relatively higher and longer period of viability observed in the in vitro germination method as compared to in the in vivo method would possibly be due to high relative humidity. This is supported by the requirement of high relative humidity for retaining viability in tri-nucleate pollen grains (Johri and Vasil, 1961; Shivanna and Johri, 1989). On the same basis, quick loss of viability in pollen from harvested flowers can be attributed to a lower level of relative humidity.

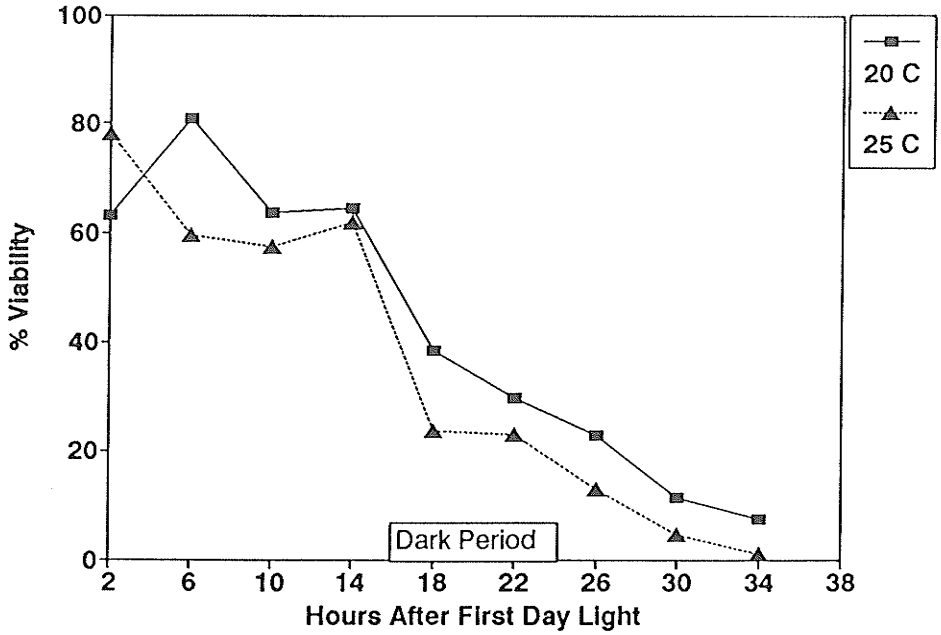

Fig. 3.7 Viability of buckwheat pollen at two temperature regimes as determined by in vivo germination method.

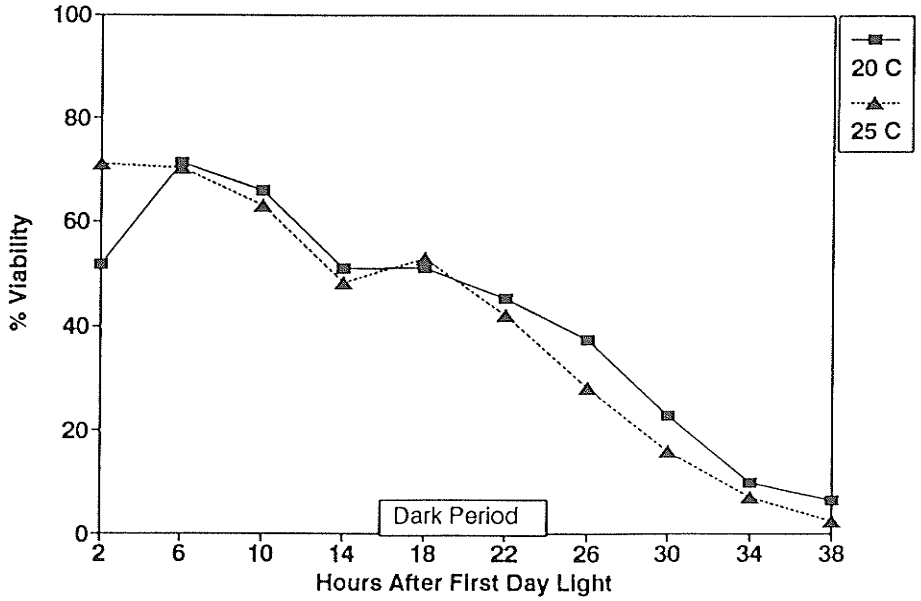

Fig. 3.8 Viability of buckwheat pollen at two temperature regimes as determined by in vitro germination method.

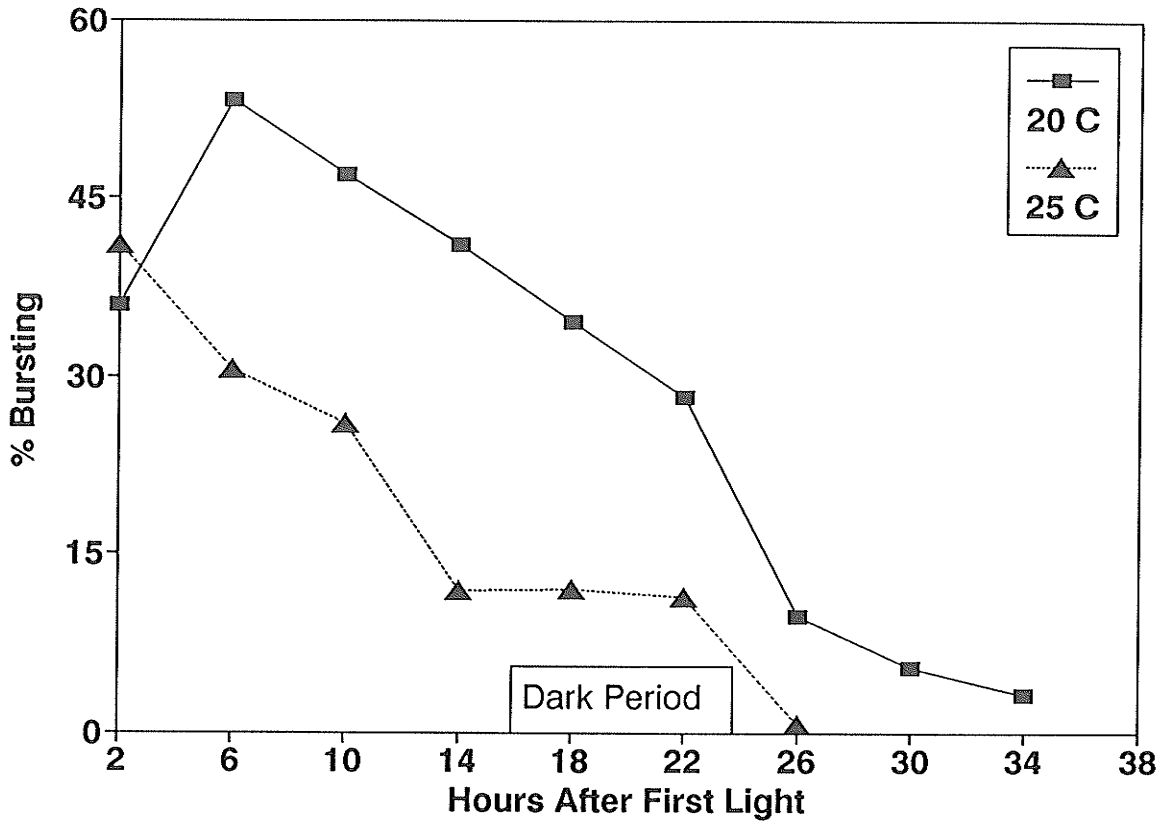

Fig. 3.9 Bursting of buckwheat pollen grain in a medium containing 0.075 % each of $MnSO_4$ and KNO_3, 0.15% $Ca(NO_3)_2.4H_2O$, 0.04 % H_3BO_3 and 30 % sucrose under two temperature regimes.

Since maximum pollen germination was observed at two hours after the first light at 25 C and after six hours at 20 C, it can be concluded that low temperature delays maturity of buckwheat pollen grains, whereas high temperature enhances it. This also suggests that the optimum time for pollination in buckwheat would be in the morning hours. Other reports have also shown that the highest percentage of seed set was obtained with the pollen collected and applied immediately after the dehiscence of anthers (Namai, 1991). Morris (1947) reported that buckwheat anther dehisced soon after opening of the flowers on bright days. The effect of temperature on duration of pollen maturity has also been reported by Xu et al (1990) in wheat.

It has been reported that unpollinated buckwheat flowers die in 24 hours (Fesenko, 1990). Observation in the present study also showed that pollinated flowers were closed on the day of opening, whereas unpollinated flowers were found partly open on the second day with a few pollen grains on their anther lobes. The closure of flowers after pollination, coupled with less than 30 per cent viability on the day following flower opening indicates that the effect of such pollen grains would be negligible in pollination. In addition, a slight wind movement causes dispersion of pollen from buckwheat flower. As a result, very little pollen would be expected to remain on the flower in the afternoon

supporting the optimum pollination period in buckwheat would be during the morning hours.

Maximum foraging of honey bees in buckwheat field was observed before noon (Singh, 1950; Free, 1970) corresponding to high percentage of viable pollen found during the same time in this study. Morton (1966) found that nearly 100 per cent of the buckwheat flowers were pollinated by 9.30 a.m. suggesting pollination occurs when there is high percentage of pollen viability. Therefore, production of inviable pollen at the time of pollination cannot be considered the cause of low seed yield in buckwheat. Similar conclusions were reached by Morton (1966) where he found, out of 456 flowers observed, only one was not pollinated and 89 per cent of the pollinated flowers had pollen tube growth into the vicinity of micropyle.

Munshi (1989) reported that stigmas mature in 15-17 hours after flower opening in most cases in buckwheat. This implies that very little pollination would occur in buckwheat, firstly as foragers would not be able to pollinate the flowers that are opened on the same day and secondly, less than 30 per cent of the pollen remain viable after 24 hours of flower opening. However, in vivo germination test in this study demonstrated that the pollen can germinate on the stigmas as early as two hours after the first light, suggesting stigmas mature within two hours and pollination occurs when there is maximum

activity of pollinators.

3.6 Conclusion

A medium for the in vitro germination of buckwheat pollen grains was successfully developed for the first time that produced up to 84 per cent germination. Most of the pollen grains were found to have a tube length several times longer than the diameter of pollen itself. Germination was dependent upon the addition of PEG 20,000 and sucrose in the medium.

Buckwheat pollen remained viable for approximately 34 to 38 hours when they were not harvested from the flowers. However, the harvested pollen grains lost viability in less than an hour under room conditions. The maturity of pollen depended upon the temperature regime under which it was produced. Maximum pollen viability was observed after two hours of day light at 25 C, whereas it took four hours to obtain the same level of viability at 20 C. No adverse effect of temperature was found on pollen viability within the experimental temperature range.

Since different genotypes may respond differently to temperatures and the temperature range in this study was very narrow, future research could be aimed at examining the effect of various temperatures on different genotypes.

4. POLLINATION BIOLOGY OF BUCKWHEAT

4.1 Abstract

Buckwheat is an obligate cross pollinated crop that has heteromorphic, sporophytic self-incompatibility system. A study to assess the extent of natural outcrossing was, therefore, conducted at Morden and Portage la Prairie in 1990 and 1991, utilizing a semi-dwarf character controlled by a single recessive gene as a marker. The semi-dwarf genotype was grown in 100 m rows running in four directions at 90° from a central 36 (6x6) m^2 plot of a normal tall genotype. At maturity, seed samples were taken from the semi-dwarf population at designated intervals ranging from 0 to 100 m beginning at the nearest point from the normal tall genotype. At least 200 F1 progenies from each sample were grown in a green house and the proportion of tall plants to semi-dwarf in the population was used to determine the percentage of outcrossing. Approximately 50 per cent outcrossing occurred where the semi-dwarf plants were immediately next to the normal plants. It then decreased with increasing distance from the central plot. However, outcrossing occurred throughout the experimental range with intermittent low and high frequencies. No significant difference was found after 9 m distance from the central plot. This suggested that pollen contamination in buckwheat is not only a function of distance between the two genotypes, but also a function of the

foraging behaviour of the pollinators and their flying pattern. Lack of directional influence in outcrossing and a sharp reduction in pollen flow within a 3 m distance from the normal plants indicated that wind was not a major factor in dispersing buckwheat pollen over long distances. An isolation distance of 100 m was not sufficient to prevent cross pollination and further research is required to determine an adequate isolation distance in buckwheat.

4.2 Introduction

Buckwheat has a very effective mechanism of outcrossing due to its heteromorphic, sporophytic self-incompatibility system. Although pollination in buckwheat is mostly entomophilous, Marshall (1969b) observed that wind was also responsible for some pollination in controlled conditions. He found that when insects were precluded from the experiment, plants grown one foot away from a wind source had 53 seeds per plant, whereas those grown 11 feet away had only 12 seeds per plant suggesting the closer the plants to a wind source, the greater the pollen distribution over distance. Krotov (1963) reported that wind accounts for approximately 20 per cent of pollination in buckwheat.

In every crop breeding program, maintenance of varietal purity is a primary concern. In self incompatible species

such as buckwheat, deterioration of varietal purity due to inter-varietal pollination is very common. In order to maintain many breeding lines and preserve the genetic resources of such crop, certain isolation distance between lines must be maintained. The size, shape and spatial arrangement of the plots are as much important as the isolation distance (Bateman, 1947a). Thus, finding a suitable isolation procedure is a prerequisite for breeders and seed certifying agencies (Bateman, 1947b). The isolation strategy of a species depends upon many factors such as breeding behaviour, pollinating agencies, buoyancy of pollen, foraging behaviour of pollinators and concentration and the longevity of pollen (Bateman, 1947a; Bradner, et al, 1965; Waser and Price, 1983).

Pollen flow can be measured by monitoring the presence of dyes or micronized powder and radio labelled pollen grain on the stigma after their application on the anther, or by analysis of progenies after crossing two genotypes with a suitable marker (Waser and Price, 1983; Handel, 1983). Among them, the most reliable method used by breeders and agronomists for many years has been progeny analysis (Handel, 1983).

There is very little information available on the extent of outcrossing in buckwheat. Due to the uncertainty of pollen

movement in buckwheat, the Canadian Seed Growers' Association has recommended an isolation distance of at least 300 m between varieties to ensure varietal purity (Anonymous, 1988). Based on the flight range of insect pollinators, Shuhua-Ren and Anlin (1986) reported that the safe isolation distance should be as far as 4 km. However, the dispersal of pollen by these pollinators and the extent of cross pollination, if any, by such pollen was not studied. Based on movement of pollen by wind, they also recommended a one km isolation distance when insects are excluded from the field. On the other hand, Krotov (1963) reported that movement of pollen by wind is limited to 5 m. Thus, there is a lack of information on the actual amount of outcrossing that occurs under field conditions. Therefore, the present experiment was designed to study the degree of natural cross pollination in buckwheat when two genotypes were grown in proximity and spaced at varying distances so as to determine the isolation distance required to prevent inter-varietal pollination in buckwheat.

4.3 LITERATURE REVIEW

4.3.1 Pollen Dispersal

Pollen dispersal occurs through many agencies including wind, water, gravity, insects, birds and animals. The most common agencies for pollinations are insects and wind. In

angiosperms, biotic pollination is the most common phenomenon (Frankel and Galun, 1977).

4.3.1.1 Dispersal of Pollen By Insects

Waser and Price (1983) studied the dispersal of pollen over distance by using dye powders, as a pollen analogue, on Delphinium nelsonii and Ipomea agregata. They observed that dye particles were dispersed as far as the eighteenth successive flower by bumble bees foraging on D. nelsonii and to the nineteenth successive flower by humming birds foraging on I. agregata. They also found the number of particles being deposited on the receptive stigmas decreased with an increase in the number of flower visits, indicating the particles brushed off as the foragers moved from flower to flower. This suggested that outcrossing frequency decreases with an increase in the isolation distance. This hypothesis was further strengthened by a decrease in the percentage of seed set with decrease in the number of pollen grains deposited on the stigma (Namai and Oshawa, 1986). In their observations, the mean seed set in buckwheat was found to be approximately 40, 70 and up to 90 per cent when the number of compatible pollen on the stigma was one, three to five and ten or more, respectively.

Gerwitz and Faulkner (1972) labelled pollen with radio

active isotopes. They found that the amount of pollen dispersed by honey bees decreased by approximately 30 per cent for each flower they visited. However, trace amount of pollen grains were found up to the tenth successive flower visited. Thus, when flight directions are random and the distances are short, pollen transfer over distance would be minimal. Conversely, if flight distances are long and unidirectional, dispersal of pollen can occur over much longer distances.

4.3.1.2 Dispersal of Pollen By Wind

Wind pollination is a relatively passive process in comparison to biotic factors. It depends upon several factors such as the number and size of the pollen grain, flower and inflorescence structure, the stigmatic surface, spacing of compatible plants, wind speed and direction, buoyancy of pollen grain and humidity (Bateman, 1946; Whitehead, 1983). In general, small and light pollen grains can disperse readily over a wider range. In an area where there is unidirectional movement of wind, especially at flowering time, outcrossing could be high. Effective wind pollination would depend upon the viability of the pollen, receptiveness and intercepting capacity of the stigma and geographical location (Whitehead, 1983). The frequency of pollination by wind increases with an increase in latitude and altitude. Wind pollination is rare in tropical environments, especially in low land and rain

forest areas, whereas it is common in temperate, deciduous and boreal forest (Whitehead, 1983; Regal, 1982).

Anemophilous plants usually have a large number of light pollen grains with smooth dry surface, whereas entomophilous species have few heavy pollen grains with sticky surfaces (Bateman, 1946; Frankel and Galun, 1977; Whitehead, 1983). Thus, pollen grains from wind pollinated species can be disseminated to longer distances as compared to pollen from insect pollinated species. It has been suggested that to pollinate a stigma with a one mm^2 area, the atmospheric concentration of pollen grain should be approximately one million for every m^2 (Proctor and Yeo, 1973), indicating wind pollinated species should produce a large number of pollen grains.

4.3.2 Foraging Behaviour of Insect Pollinators

The behaviour of pollinators is likely to depend on the availability of nectar and pollen. Moffetti et al (1976) observed that cotton genotypes with high sugar concentration in floral nectar attracted more honey bees than genotypes with low sugar concentration. The number of foragers was found to be doubled when sugar concentration was higher by 10 per cent, whereas no differences in insect visit could be detected when the sugar concentration was lower than three per cent. The

sucrose concentration in buckwheat nectar ranges from 62.7 to 76.2 per cent (McRory and Jay, 1970) as compared to 22.6 to 38.7 per cent in cotton (Moffetti et al, 1976) and 51 per cent in Brassica rapa (Free, 1970). This suggests that more pollinators should be attracted to buckwheat flowers than to cotton and rapeseed. Moffetti et al (1976) found a seasonal and hourly variation in the quantity of nectar secretion within the same genotype of cotton. The nectar secretion increased steadily from 9 a.m. with a peak being reached at 5 p.m. and decreased secretion thereafter.

Marden and Waddington (1981) investigated the visiting pattern of honey bees (Apis mellifera) on artificial flowers. They found that when two flower colors with equal sugar concentration were placed equidistance, bees maintained a high degree of flower constancy to one flower color. However, when the flowers were kept at different distances, they visited the closest flower most frequently, indicating that when given a choice they switched to the closest reward to save their flight cost. Their finding partly supports the 'theory of flower constancy' in honey bees (Grant, 1950; Free, 1963) which states that an individual always intends to visit a single flower species for pollen and/or nectar. Honey bees show more flower constancy than bumble bees (Bombus species) followed by solitary bees (Grant, 1950; Wiser, 1983).

Woodell (1978) reported influence of wind direction on the movement of bumble bees foraging on Armaria meritima and Limonium vulgare. Bumble bees chose to fly with direction of the wind. Because of the directionality, the chance of revisiting the same spot on a single foraging trip was lessened, indicating outcrossing over distance would decrease steadily with an increase in distance. Bumble bees carried a considerable amount of pollen on their body and frequently brushed off the pollen as they foraged on new flowers. However, they did not sweep the pollen in their curbiculae each time they visited a flower. As a consequence, pollen dispersal would be expected to remain persistent over a long distance.

Bumble bees were found to fly short distances of approximately 20 cm after foraging in nectar rich flowers, but their flying distance nearly doubled after foraging in nectar poor flowers (Waddington, 1981 as cited by Namai, 1991). This suggests a longer distance of pollen dispersal from nectar poor flowers than those from nectar rich flowers.

4.3.3 Pollinators of Buckwheat

Flowering in buckwheat is a continuous process due to its indeterminate growth habit. The nectaries of the flowers, located at the base of the stamens, attract different insect

pollinators, especially common bees. When the nectar gatherers feed in the nectaries, they are easily charged with pollen (Free, 1970). Thus, they can effectively transfer pollen as they forage from one flower to another. Ren-Shuhua and Liu (1986) observed the variety of insects involved in buckwheat pollination. They found two orders, 10 families, 17 genera and 37 species of pollinators in a buckwheat field in Inner Mongolia, China. The dominant pollinators, constituting about 65 per cent of the total pollinators, were Apoidae including honey bees, bumble bees, ardenid bees and syrphidae. They were found to collect nectar as well as pollen when both were available.

McRory and Jay (1970) and Smirl (1970), in their preliminary work on buckwheat pollinators, found common bees to be the most prevalent pollinators. Other species involved were diptera (flies), homoptera (mostly leaf hoppers), hemiptera (mostly plant bugs) and neuroptera (lace wings). Free (1970) reported that honey bees constituted 63-72 per cent of the insects that visited buckwheat flowers. Heinrich (unpublished paper) reported that bumble bees can work through out the day from dawn to dusk. They can forage at a temperature as low as zero C and are found not only in warm regions, but also in the Arctic. In warm areas, another important pollinator of buckwheat is the alfalfa leaf cutter bee (Megachille rotundus), which cannot forage at temperature

below 70 F (Hobbs, 1967).

4.3.4 Foraging Behaviour of Pollinators on Buckwheat

Singh (1950) observed the highest activity of honey bees between 10 a.m. to 1 p.m. in buckwheat fields. Before noon, the activity of a single bee was concentrated in a small area due to the abundance of nectar. As the amount of nectar decreased in the afternoon, the bees hovered around many flowers over a large distance, suggesting that nectar flow was the major factor regulating bee activity.

Honey bees are attracted to the buckwheat field as they can readily recognise the odour of the nectar. It was found that one honey bee visited an average of 14 flowers per minute and worked for 4-5 hours making approximately 5 trips in a day (Free, 1970). They visited the flowers most frequently from 9 a.m. to noon. The quantity of nectar production was found to increase during the late a.m. and early p.m. and continued to be produced till 3-5 p.m. (Smirl, 1970; McRory and Jay, 1970). The concentration of sucrose in nectar usually increased as the afternoon progressed, possibly due to evaporation of its moisture content.

McDonald (1964) reported that honey bees had the tendency of collecting nectar from a single species in a given day.

They continued to extract nectar from the same flower as long as the nectar was available. Upon depletion of nectar in the afternoon, the bees were annoyed and spent the rest of the day in the hive. He further reported that the buckwheat flowers yield nectar most abundantly during the morning hours and observed a large number of bees working in buckwheat field during the same period and only few, if any, in the afternoon.

4.3.5 Pollen Dispersal and Isolation Distance in Buckwheat

Based on the distance pollinators travelled, Shuhua-Ren and Anlin (1986) recommended an isolation of 4 km as the safest distance for preventing varietal contamination in buckwheat. They observed that most of the pollinators of buckwheat travelled within a 500-1000 meter radius. At 2 km, the frequency of insects decreased almost three fold. Although the number of insects declined as the distance from their release increased, bumble bees flew as far as 4.5 km followed by honey bees who reached 4.0 km. This suggests that bumble bees have a greater ability to fly and pollinate buckwheat flowers at a greater distance than do honey bees.

Wind movement of pollen was monitored by placing vaseline coated slides at different distances from a buckwheat field (Shuhua-Ren and Anlin, 1986). They reported that the dispersal distance of pollen was 500-600 m and 1,000 m at low

(2.3-3.2 m/s) and high wind speed (6.3 m/s), respectively. However Krotov (1963), in a similar experiment, reported that the movement of pollen by wind was limited to 5 m at low wind speed. He observed a 50 per cent reduction in pollen concentration when the distance was increased from 0.5 to 1 m from the pollen source.

Namai (1990) reported no cross pollination effect between diploid and tetraploid buckwheat genotypes. Therefore, a tessellated plot design of diploid and tetraploid has been practised in the CIS and Poland to give isolation for seed multiplication. Marshall (1980) separated diploid strains with 5-row strip (1.5 m wide) of sorghum and sudan grass hybrids planted at the same time as buckwheat to prevent varietal contamination.

4.3.6 Factors Affecting Extent of Outcrossing

4.3.6.1 Distance and Direction

The effect of distance on outcrossing has been reported by several authors (Crane and Mather, 1943; Bateman, 1947a; Afzal and Khan, 1950; Fryxell, 1956; Bradner et al, 1965; Datta et al, 1982; Rai and Jain, 1982;). In all their experiments, they found a regular and rapid decrease of inter-varietal crossing as the two varieties were spaced further

apart. Afzal and Khan (1950) studied the degree of natural outcrossing in cotton in different directions and distances by monitoring the outcrossing rate in a large field up to a distance of two miles away from the exotic pollen source. A negligible amount of pollen contamination was recorded after 12.5 feet from the contaminating pollen source and none were found beyond 100 ft. On this basis, they recommended that the safest isolation distance to grow cotton as a seed crop would be 100 ft. They did not find any influence of wind direction and velocity on varietal contamination.

Datta et al (1982) investigated natural outcrossing in jute (Corchorus olitorius L.) in two planting arrangements. In one experiment, a cultivar with a dominant marker gene was planted at the centre and surrounded by another cultivar with a recessive marker. In another design, the planting arrangement was reversed. Maximum outcrossing, in both designs, was found at the minimum distance from the pollen source and decreased with an increase in distance. Outcrossing was not found to occur beyond 9 m from the pollen source. Non-significant effect of interactions between direction and distance, year and distance and year and direction led them to conclude that the distance between the two cultivars was a consistent factor of outcrossing and wind had no role influencing varietal contamination in jute.

Bateman (1947a) studied outcrossing frequency in radish (Raphanus sativus L). He found that pollen contamination decreased from 60 per cent to 13 per cent as the distance was increased from 20 ft. to 80 ft. from the source of pollen, but it was still 6 per cent at 140 ft. After increasing the isolation distance from 160 ft. to 580 ft., the reduction in frequency of outcrossing was too small to detect that remained in the neighbourhood of one per cent. Fryxell (1956), on the other hand, found a steady reduction in the degree of pollen contamination in cotton as the distance between two varieties increased.

4.3.6.2 Varietal Mass

Fryxell (1956) examined the proportion of varietal contamination in cotton by growing two varieties in parallel rows. The frequency of outcrossing was higher in the samples taken along the border edges of the field than those taken in the interior portion. In the former, the frequency was quite high at 165 ft from the source of foreign pollen, but in the latter, it decreased to a negligible amount by 50 ft. He attributed this difference to the effect of varietal mass and concluded that outcrossing frequency was higher when the plant population was low and vice versa.

Similarly, Crane and Mather (1943) studied natural cross

pollination on radish by planting two varieties in different patterns. In densely planted experiments, they found 30-40 per cent outcrossing between adjacent rows of the two varieties that decreased to almost one per cent as the distance between the varieties increased to 15 feet. However, in a sparsely planted experiment, outcrossing persisted as far as 95 feet from the contaminant pollen source. They observed that when there was an abundance of flowers, i.e, a large plot with a dense population, the foraging area of the bees was found to be confined to a small area. On this basis, they recommended that if varieties were grown in a large area, an isolation distance of 300 feet was enough to maintain varietal purity in radish. However, if the crop was grown in a smaller area, a greater isolation distance between varieties was necessary.

4.3.6.3 Type of Insect Pollinators

Bradner et al (1965) observed natural outcrossing in alfalfa by monitoring the foraging behaviour of insect pollinators. They concluded that the degree of outcrossing was a function of the foraging species, distance from the foreign pollen source and the season. The effect of honey bees and alfalfa leaf cutter bees on outcrossing was observed to be 6.5 per cent at 200 m, whereas approximately the same amount of outcrossing occurred as far as one mile from the

pollen source when bumble bees were involved in pollination. This indicates that bumble bees carried pollen for longer distance than did hive bees. Wasps, Bembix occidentalis, also erratically visited flowers to collect nectar over a wide distance. But, their effect as pollen carriers was negligible since they lack a pollen carrying device and enough body hairs to effectively collect the pollen. Fryxell (1956) reported that the random outcrossing frequency in cotton was due to the indiscriminate foraging activities of the insect pollinators.

Crane and Mather (1943) observed that when bee hives were placed close to radish plots, intercrossing became less regular and spread up to 240 ft from the foreign pollen source, indicating that the greater the number of pollinators per unit area, the further the pollen was dispersed. They also concluded that the foraging area of a honey bee during anyone visit was less than 4-5 yards. Butler et al (1943) found similar results and reported that the effective foraging area of a honey bee was generally confined to an area of 4-5 yards in diameter.

4.3.6.4 Planting Design and Field Shape

An influence of planting design and field shape on pollen contamination has been reported. Afzal & Khan (1950) found that outcrossing in cotton was negligible at 40 feet from the

source of foreign pollen when the allied varieties were grown as barriers, while a trace amount of outcrossing occurred up to 75 feet from the pollen source in an open space. Rai and Jain (1982) planted barley (Avena batata) in a cross and spiral pattern with a dominant marker gene at the centre. They found more pollen contamination in the cross shaped design than in the spiral design. Similarly, Pedersen et al (1969) reported a higher frequency of outcrossing in a rectangular plot of alfalfa than in a square plot under the same management conditions.

4.4 MATERIALS AND METHODS

The experiment was conducted in two locations at the Agriculture Canada Research Stations at Portage La Prairie and Morden, Manitoba in 1990 and 1991. Two phenotypically different lines, G410 and Mancan, homozygous for different plant stature were used in the study (Fig.4.2). The semi-dwarf character of G410, inherited by a single recessive gene (Campbell, 1987) was utilized as a marker. A 203 X 203 m plot was assigned for the experiment. The normal, tall statured cultivar, Mancan, was planted in a central 36 m^2 area of the field in 20 rows 6 m long and with a 0.3 m row spacing (Fig 4.1). A single 100 m row was planted with G410 in all four directions beginning immediately after the outermost row of the central plot in such a way that the semi-dwarf rows were

perpendicular to the central plot. To avoid missing plots, which occurred due to poor germination in 1990, two adjacent rows 0.3 m apart of the semi-dwarf were established in 1991.

The experiment at Portage la Prairie was planted in an open field in 1990. All other experiments were planted within wheat fields that were plowed down at the time of the commencement of buckwheat flowering. Pollination was allowed to occur through the natural agencies of insects and wind. Although flowering in Mancan began five days earlier than that of G410, pollen was available for transfer throughout the growing season due to the indeterminate growth habit of buckwheat.

After the first occurrence of frost, seed samples were taken from the semi-dwarf at specified distances beginning at the nearest point from the central plot. Samples were taken every 3 m for the first 30 m, every 5 m from 30 m to 60 m, and every 10 m thereafter. Thus, 21 samples were collected from each arm designated as east, west, north and south. At least 200 F1 progenies from each sample were grown out in a green house under a 16 hour day length. The plants were rated at the 3-4 internodal stage and classified as to being normal tall or dwarf (Fig. 4.3). The proportion of plants exhibiting a tall stature in the F1 population was used to determine the percentage of outcrossing. A total of 67,216 plants were

rated to assess the degree of natural outcrossing. In this paper, the term 'outcrossing' or 'pollen contamination' is used to designate cross pollination of the recessive semi-dwarf population with pollen from the dominant normal tall cultivar.

Analysis of variance was performed in a randomized complete block design considering each site as a replication to determine the effect of direction and distance in outcrossing. The outcrossing percentages obtained at each distance in all four directions within the replication were pooled and regression analysis was performed to predict the outcrossing percentage at a certain distance within the experimental range. The outcrossing percentage was transformed into a log scale at the base of 10 before the regression analysis.

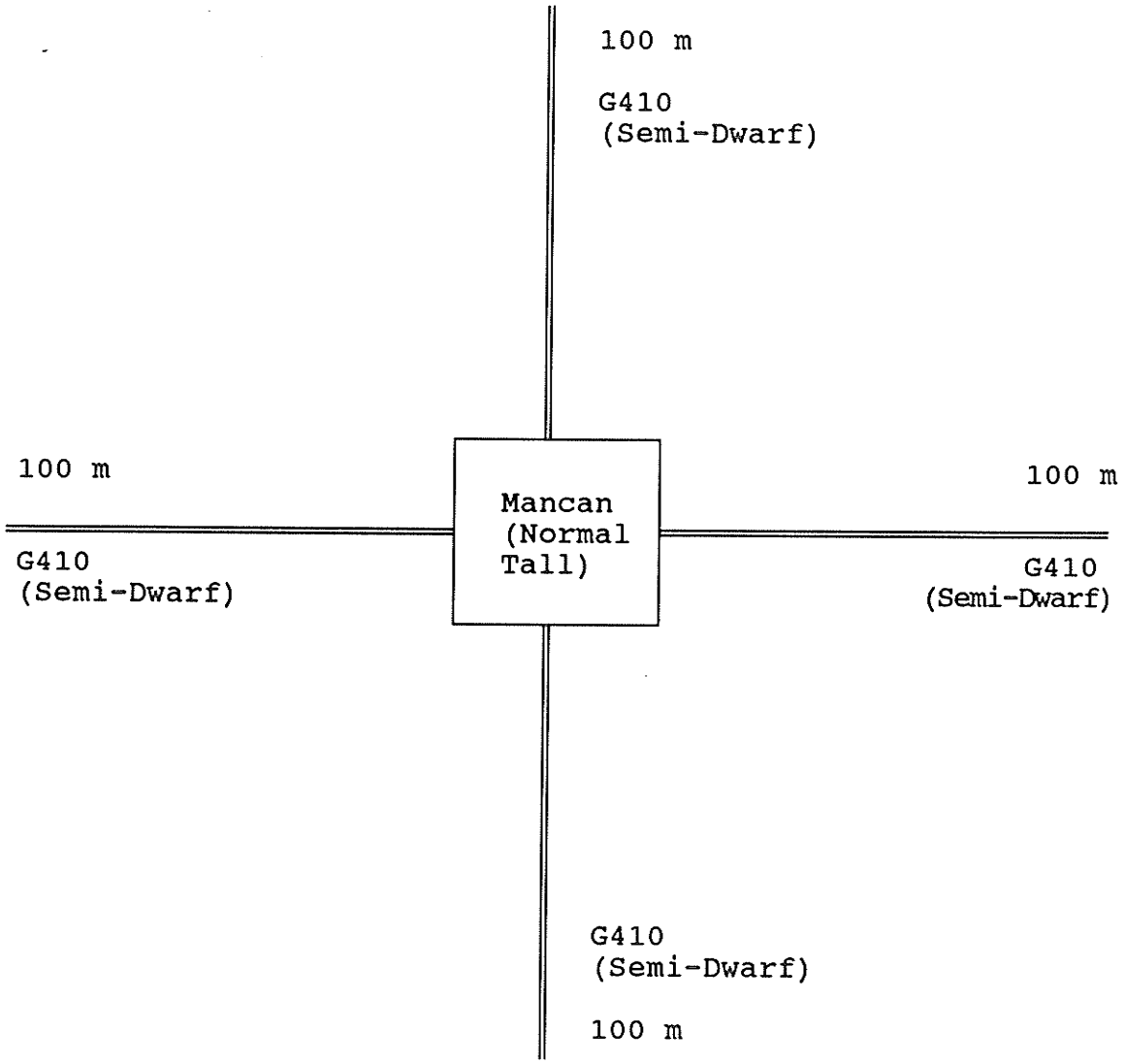

Fig. 4.1 Layout of buckwheat outcrossing experiment conducted at Morden and Portage la Prairie, Manitoba in 1990 and 1991.

Fig. 4.2 Semi-dwarf (G410) and normal tall (Mancan) genotypes used in the determination of the outcrossing in buckwheat.

Fig. 4.3 F1 progenies from field sampling showing normal tall and semi-dwarf plants.

4.5 RESULTS AND DISCUSSION

The analysis of variance shows that the distance from the normal tall variety had highly significant effect on the degree of outcrossing, while direction and interaction between distance and direction had non-significant effect (Table 4.1). This indicated that a random nature of outcrossing occurred over all directions and distance alone was the major factor contributing to outcrossing. Approximately 50 per cent outcrossing was found to occur at the adjoining rows of two genotypes. In the beginning, outcrossing decreased rapidly with an increase in the distance from the central plot. However, it persisted throughout the 100 m row with intermittent low and high frequencies (Fig 4.4).

Table 4.1 Analysis of Variance for Outcrossing Percentage in Buckwheat Grown at Morden and Portage la Prairie, Manitoba in 1990 and 1991.

Source	df	MS	F	Prob > F
Replication	3	140.55	4.67	0.0034
Direction (A)	3	48.15	1.60	0.1901
Distance (B)	20	1435.30	47.68	0.0001
A*B	60	24.46	0.81	0.8292
Error	237	30.10		
Total	323 [1]			

[1] 13 observations had missing values.

Bateman (1950) has reported that the flight length of insects and dispersal of pollen by insects can best be described by leptokursis distributions. The plotted means of outcrossing frequency against the distance in present study indicates a similar trend (Fig.4.4). No detectable decrease in pollen contamination was found throughout the experimental range after 9 m distance from the central plot (Table 4.2). Similar results were obtained by Bateman (1947b) in radish where outcrossing did not decrease appreciably by increasing the isolation distance from 160 to 580 feet.

The decrease in outcrossing frequency with an increase in isolation distance was described by a quadratic model (Fig. 4.5). The frequency of outcrossing was transformed into a log scale to linearize the data. This model could describe only less than 50 per cent of the variation. A low r^2 value (0.44) might be due to random low and high frequency of outcrossing.

The pollen grains carried by insects were subject to replacement by pollen of another flower as they move from flower to flower during foraging (Bateman, 1947b; Free, 1970). Therefore, the proportion of pollen contamination should decrease with increase in distance. Afzal & Khan (1950) observed similar results in cotton and concluded that outcrossing does not occur beyond 100 ft. Similar results were obtained by Bradner et al (1965) in alfalfa and Datta et

Table 4.2 Duncan's Multiple Means Separation Test Results for Buckwheat Overall Mean Outcrossing Percentage.

Distance (m)	Mean Outcrossing (%)
0	50.22 a *
3	11.82 b
6	6.68 cd
9	5.96 cd
12	4.83 cde
15	2.93 cde
18	4.77 cde
21	5.00 cde
24	4.67 cde
27	3.99 cde
30	4.76 cde
35	5.10 cde
40	2.05 de
45	3.75 cde
50	7.20 c
55	3.66 cde
60	2.70 cde
70	2.09 de
80	1.03 e
90	2.78 cde
100	0.74 e

* Means followed by the same letter are not significantly different at 0.05 probability level.

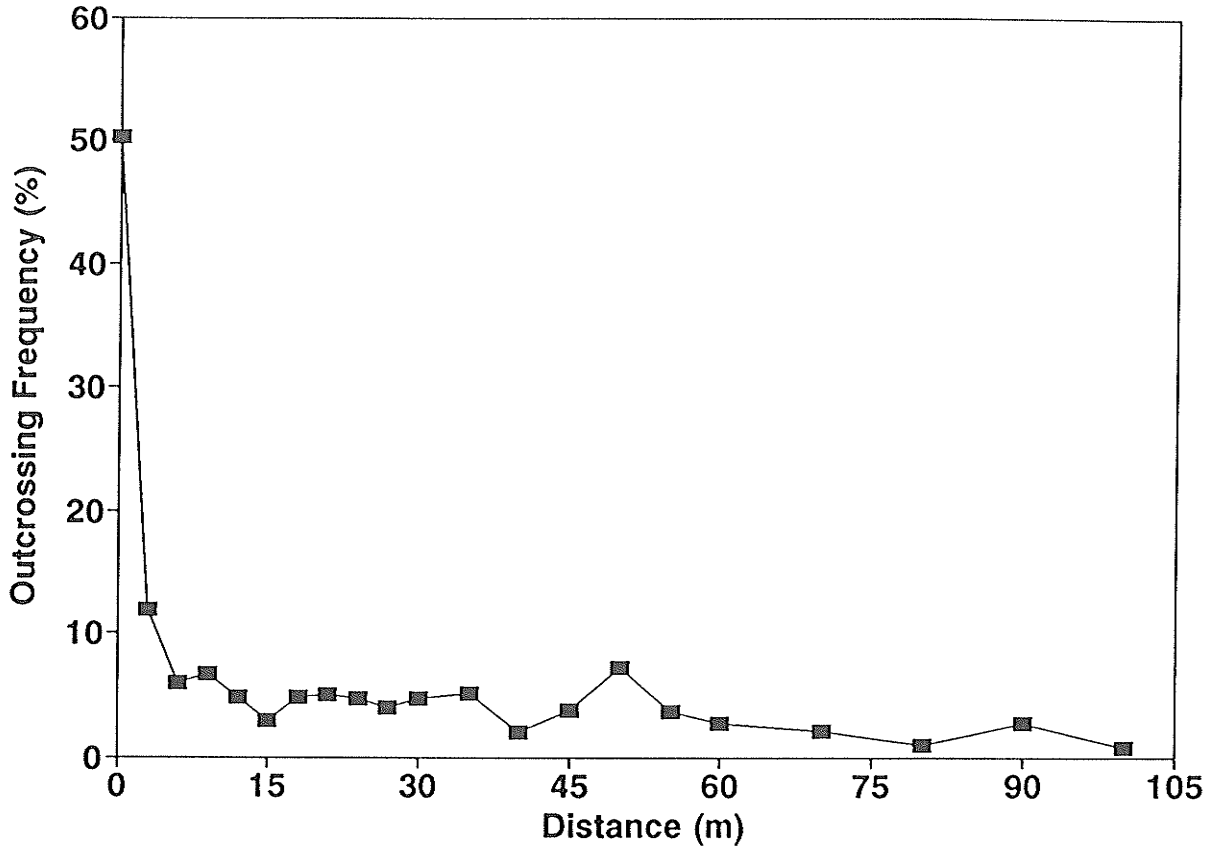

Fig. 4.4 Mean outcrossing percentage of buckwheat averaged over directions and replications grown at Morden and Portage la Prairie, Manitoba in 1990 and 1991.

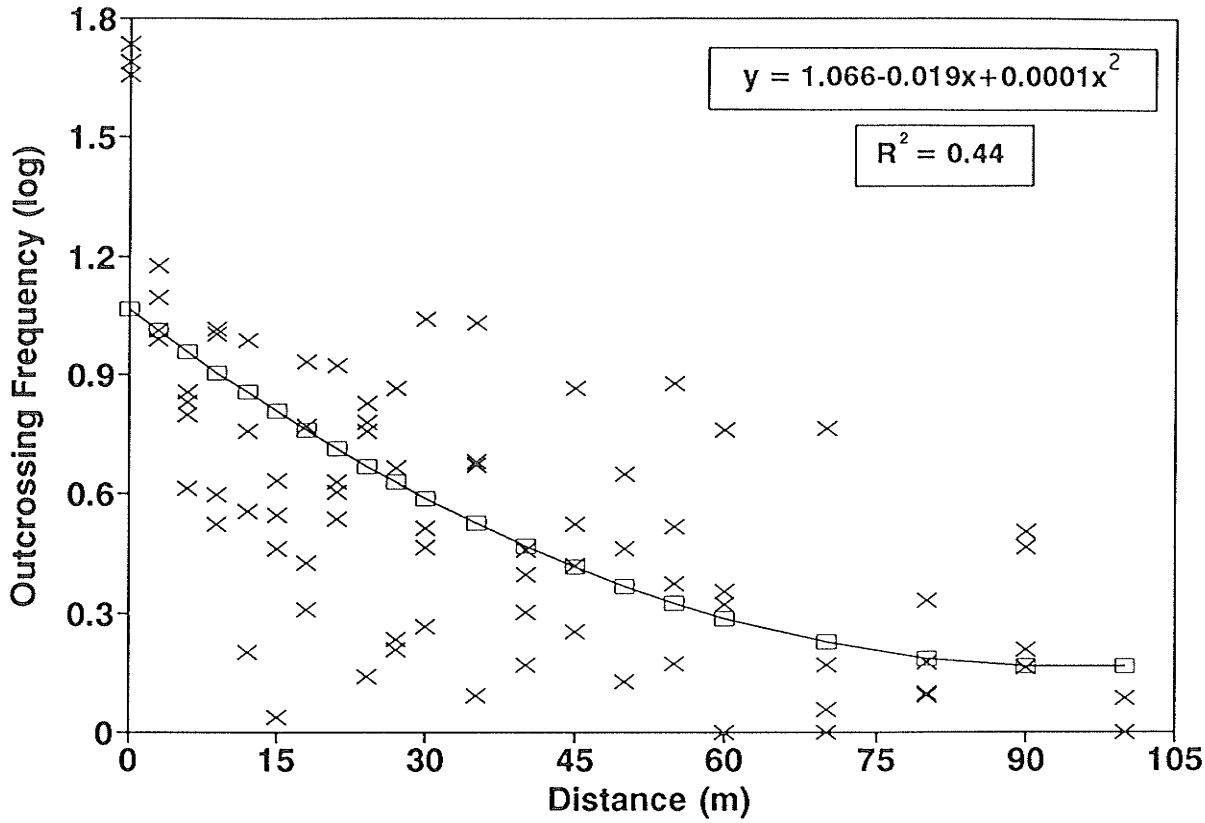

Fig. 4.5 Semi-logarithmic regression line of buckwheat outcrossing percentage on averaged data over directions after transformation of the percentage into a log scale.

al (1982) in jute. In the present study, approximately 7 per cent pollen contamination even at a distance of 50 m (Table 4.2) indicates that at least some insect pollinators flew long flight distances and carried a large amount of pollen with them. This supports the work of Bateman (1947b) where he observed occasional random flight of hive bees, solitary bees and hover flies over directions that visited the same plant at irregular intervals. Similar conclusions were reached by Fryxell (1956) in cotton.

It appears that the foragers concentrated their activity wherever they first landed as long as the nectar was available. This agrees with the findings of Butler et al (1943) where they reported that honey bees concentrate their activity in less than a five-yard diameter. After depletion of nectar, they went back to their hives or made exploratory flights rambling all over the field. The latter phenomenon must have occurred to produce random high frequency of outcrossing in this experiment. Singh (1950) observed that the bees visited flower in limited areas in the morning, however, as the availability of nectar was reduced in the afternoon, they wandered over a larger area. Thus, a high level of outcrossing was expected wherever they landed directly from the central plot.

Most of the insect pollinators, including honey bees,

take short flights, but now and then, they soar up rapidly from the plant and forage elsewhere (Bateman, 1947b; Singh, 1950). This normally happens when the nectar and/or pollen is depleted from the flower. Bumble bee and some solitary bees can carry more pollen grains on their body (Free and Williams, 1972; Woodell, 1978) and can travel longer distances (Bradner et al, 1965; Shuhua-Ren and Anlin, 1986) as compared to honey bees, suggesting that when they are present, outcrossing can be expected to occur over a greater distance. Thus, relatively high outcrossing even at a distance of 90 m from the pollen source possibly indicates the involvement of these foragers in the present study.

The optimum foraging theory states that the flight distance between the successive flower visits would be small if the density of plants is large and vice versa (Levin, 1978). Upon the availability of enough reward, the pollinators would fly short distances between successive flower visits to minimize their flight cost (Waser and Price, 1983). This implies that when there is a dense population, flying distance should be small and so would be the outcrossing frequency over distance. Similar results have been reported by several investigators where they found the larger the mass of the pollen recipient genotype, the lower the outcrossing frequency (Crane and Mather, 1943; Afzal and Khan, 1950; Fryxell, 1956). In this study, there was very low

plant density. As a result, the frequency of outcrossing that occurred over distance might have been higher than what would be expected in a larger field. Thus, there might be a possibility of lowering the frequency of natural outcrossing over distance by increasing the varietal mass.

Movement of pollen occurs not only as the foragers move from one flower to another, but also through their mutual contact as they brush against each other when returning to a colony (Free and Williams, 1972; Degrandi-hoffman et al, 1986). This suggests the possibility of cross pollinating a flower by a forager that had never visited that species before. The present study showed that harvested buckwheat pollen normally loses viability in less than an hour and, therefore, the role of mutual contact may not be crucial in promoting cross pollination in buckwheat. However, the effect of micro-environment on pollinators' bodies on the longevity of buckwheat pollen is not known. The in vitro pollen germination technique developed in this study would be a very useful means in conducting further research in this area.

The sharp reduction in frequency of pollen transfer with an increase in distance from zero to 3 m from the pollen source and the nonsignificant effect of direction on outcrossing suggests that wind was not a factor for carrying buckwheat pollen for long distances. Since pollen dispersal

by wind would be expected to take place in a downwind direction, the direction downwind of the prevailing winds should show the highest degree of outcrossing (Frankel and Galun, 1977; Datta et al, 1982) if wind is an important factor. No effect of direction was found in this study indicating wind had little or no influence in dispersal of buckwheat pollen, which is similar to those results obtained by Afzal and Khan (1950) in cotton and Datta et al (1982) in jute. This result also agrees with the findings of Marshall (1969b) where he found seed set in buckwheat was reduced very rapidly when the plants were grown at 11 ft as compared to 1 ft from the wind source. Krotov (1963) did not find any pollen movement by wind beyond 5 m in the buckwheat field.

Before recommending an isolation distance for any crop species, a permissible level of contamination must be determined based upon the breeding system of crop and the use for which the seed is intended (Bateman, 1946). Since buckwheat is an obligate outbreeder, a low amount of contamination might be permitted in a seed lot as there is already naturally occurring genetic variability within a variety. However, the standards set by the Canadian Seed Growers' Association (Anonymous, 1988) show the same level of impurity for self pollinated as well as for cross pollinated crops. Maximum impurity tolerance for foundation and registered buckwheat seed is one in 10,000, and for certified

seed is five in 10,000 seeds. The present study showed approximately 5 per cent outcrossing after a distance of 9 m from the contaminating pollen source and this frequency was found to not differ significantly over the entire range of 100 m. Therefore, a 100 m isolation distance would not be adequate to prevent inter-varietal contamination in buckwheat.

4.6 Conclusion

The sharp reduction observed in inter-varietal contamination within the first 3 m distance from the source of foreign pollen and the lack of directional influence on cross pollination suggests that wind does not carry buckwheat pollen for long distances. The intermittent low and high frequency of outcrossing observed over a large range indicates that the extent of pollen contamination in buckwheat was not only a function of distance between the two genotypes, but also a function of the foraging behaviour of the pollinators and their flying pattern. Successive increases in isolation distance became less and less effective in decreasing the degree of outcrossing as compared to the first few meters. This coupled with the occurrence of outcrossing over the entire range of 100 m distance suggested that insect pollination is the major factor involved in the cross pollination. Further research is required to determine the optimum isolation distance for buckwheat.

5. DETERMINATION OF YIELD IN BUCKWHEAT

5.1. Abstract

Ten buckwheat germplasms of diverse origin were evaluated at the Agriculture Canada Research Station, Morden, Manitoba in 1990 and 1991 to elucidate the relationships among grain yield, yield components and other important agronomic traits. Each entry was sown in a four replicate randomized complete block design in both years. Observations on grain yield, yield components and important agronomic characters were taken for each genotype. A wide range of variation was observed among the genotypes for the various traits. Correlation values for most traits were found to be low and inconsistent due to the high degree of environmental influence. Seeds per plant, seeds per inflorescence, plant stand and duration from flowering to maturity were consistently correlated to grain yield over years. The length of pre-flowering period was inversely related to the duration from flowering to maturity in both years. The number of branches was positively correlated to the number of inflorescences, while a negative correlation was found between the number of inflorescences and the number of seeds per inflorescence. An increase in yield of buckwheat could be accomplished by selection for lower number of branches, increasing the number of seeds per plant and by shortening the pre-blossoming period through selection

for a longer duration from flowering to maturity.

5.2. Introduction

The objective of plant breeding program is to increase the value of desirable traits. The increase of yield potential has always been of fundamental importance in plant breeding programs. Grain yield of any crop species is a complex trait that depends upon several factors (Grafius, 1964; Durate et al, 1972). Improvement of yield is generally accomplished by selecting desirable plants in a heterozygous population. Selection of individual plants in early generations requires the use of a model that can be utilized as a substitute for the direct measurement of grain yield (Anderson, 1986). Characters such as number of inflorescence m^{-2}, number of grains per inflorescence and the weight per grain have been regarded as the yield components that have the greatest influence on yield in many crop species. Various components of yield along with important physiological and morphological characteristics that are beneficial under specific environmental conditions have been presented in crop models by several authors (Donald, 1968; Rasmusson and Cannell, 1970; Zuberi and Ahmed, 1973; Mock and Pearce, 1975; Adams, 1982; Rasmusson, 1987).

The seed yield of any crop plant is the combined result

of many growth processes that are ultimately expressed in yield components (Westermann and Crothers, 1977) indicating that yield might be improved by selecting for individual components of yield. In the past, however, components of yield as selection criteria have not been extensively used due to the high degree of environmental influence (Grafius, 1964; Rasmusson and Cannell, 1970; Frey, 1971). Moreover, there are often negative correlations among yield components that negate selection for an increased value in one trait (Adams, 1967; Coyne, 1969; Rasmusson and Cannell, 1970).

Due to compensation mechanisms, symmetry in the size of plant parts and pleiotropic effects among yield components, development of a plant model that gives maximum yield in a given environment is difficult (Adams, 1967; Adams and Grafius, 1971; Grafius, 1978; Rasmusson, 1987). It is necessary, therefore, to balance the value of yield related traits so that maximum yield can be realized. Elucidation of the interrelationships among yield components is necessary in order to determine the best combination of traits for the maximization of yield (Westermann and Crothers, 1977).

Very little information is available regarding the relationship of yield components to one another and to grain yield in buckwheat. Therefore, the present experiment was designed to elucidate some of the interrelationships of the

various parameters that affect seed yield in buckwheat.

5.3. LITERATURE REVIEW

5.3.1. Components of Yield

Yield components consist of the major traits contributing to grain yield. They differ from species to species depending upon the nature of the crop. Grain yield in cereals is composed of the number of spikes per unit area, the number of kernels per spike and individual kernel weight (Grafius, 1964; Bulman and Hunt, 1988; Chapko and Brinkman, 1991). In legumes, it is the number of plants per unit area, the number of pods per plant, the number of seeds per pod and the mean seed weight (Zimmerman et al, 1984; Duarte and Adams, 1972; Westermann and Crothers, 1977). In oilseed crops, such as Brassica species, it has been regarded as the number of pods per unit area, the number of seeds per pod and the mean seed weight (Zuberi and Ahmed, 1973).

It is obvious from the above examples that the number of plants per unit area, the number of seeds per plant and mean seed weight are the most important factors contributing to yield in many species. Plant stands can be increased simply by choosing the appropriate seeding rate, but an increase in seed number and seed weight requires selection pressure.

5.3.2. Relationship of Yield Components With Grain Yield

Islam and Langer (1985) observed selection for grain number per spike and per spikelet resulted in a significant increase in grain yield per plant in wheat. However, selection for larger grain weight had a small and inconsistent effect on yield increase. They concluded that selection of F2 populations for grain number per spike or spikelet is more effective than selection for seed weight.

Zuberi and Ahmed (1973) found that total seed yield per plant was positively correlated to the number of fruit per plant, the number of seeds per fruit and fruit length in Brassica campestris var. Toria. The number of seeds per fruit was also positively correlated to fruit length. Chapko and Brinkman (1991) studied the relationship of panicle weight to grain yield in oats. Panicle weight, which is made up of two of the three major yield components in oats, had a high positive correlation with spikelet per panicle but a negative relationship with the number of spikes per unit area. Pixley and Frey (1991) found a positive relationship between test weight and grain yield in oats that would facilitate breeding for high yield with a concomitant increase in test weight.

Rasmusson and Cannell (1970) studied the relationship between yield and its components in barley. They found

selection for yield through yield components was effective in some cases, but ineffective in others. On that basis, they stated that the contribution of yield components to yield was highly influenced by the environment and yield components could not be regarded as a routine criteria of selection. Rasmusson (1987) has outlined detailed studies on various traits, their genetic diversity and heritability in barley. He observed that compensation mechanism among yield components, symmetry in size among plant parts, and pleiotropic effects made it difficult to make improvements in yield through selection of yield components.

Bulman and Hunt (1988) examined the relationship between tiller and spike number and between grain yield and spike number in winter wheat. Final spike number was linearly correlated with tiller number in most cases. Likewise, grain number was linearly related to spike number over a range of 400 to 1200 m^2. They suggested that low yield in winter wheat was related to a reduced number of spikes per unit area.

Fonseca and Patterson (1968) reported a negative correlation between kernel weight and number of spikes, and kernel weight and kernel number per spike in winter wheat, suggesting little scope for yield improvement through selection of yield components. Anderson (1986) studied the relationships between yield components and grain yield in

winter wheat under optimum plant densities. He found that grain yield was positively related to the number of culms, the number of spikes and the number of seeds per unit area. However, spike size (seeds per spike or spike weight) and seed size were not consistently related to grain yield.

Knott and Gebeyehou (1987) concluded that the length of the vegetative period was inversely correlated to the length of the grain filling period in durum wheat. An inconsistent and small correlation was observed between grain yield and the length of these two growth periods indicating an optimum combination of vegetative and grain filling periods to produce maximum yield was not available in their study.

In buckwheat, large seed size was thought to be associated with high grain yield. Therefore, attention was given to breeding tetraploid cultivars. However, tetraploids were found to exhibit lower fertility than their diploid counterparts due to irregular meiosis (De Jong, 1972). Instead, diploid cultivars such as Mancan and Manor with large seed have been developed (Campbell and Gubbels, 1986) indicating little need for tetraploid breeding. Furthermore, development of large seeded tetraploid cultivars in Japan led to the reduction in groat content as compared to diploid cultivars (Campbell, 1992, personnel communications).

Traditional methods of breeding highly productive plants led to the selection of plant types with strong branching, a large number of flower clusters and tall growth habit in buckwheat (Fesenko and Martynenko, 1984). However, such plant types required more moisture due to their large canopy, became less responsive to fertilizer and were subject to lodging. These results prompted breeders to develop varieties with higher productivity without an increase in plant size. Fesenko and Martynenko (1984) studied the relationships of branching with grain yield in plants grown under high and low plant stand conditions. It was observed that approximately 70 to 80 per cent of the yield was contributed by the main stem under high stand conditions, whereas the main stem contributed less than 20 per cent under a low stand conditions. The contribution of branches to yield was found to be decreased with an increase in the number of branching under both conditions, indicating high branching as an undesirable character. They recommended selecting plants dominant with main stem development accompanied by an increased productivity of the flower buds. The presence of a negative relationship between the development of the main stem and lateral branches was found to help in the selection process, especially in determinate type where the relationship was clearly expressed.

Positive correlations were found between the number of grains per plant with various traits in buckwheat (Petelina,

1980). Grain yield per plant was found to be correlated with the number of branches of the first order (r = 0.54), the number of leaves (r = 0.54), the number of inflorescences (r = 0.71) and the number of grains per inflorescence.

Ruszkowski (1991) studied the relationship of branching to grain yield by planting buckwheat at different densities. He found that the closer the spacing, the fewer the branches and the lower the yield per plant. However, due to the increased number of plants per unit area, grain yield was higher in closely spaced planting than that of widely spaced planting. Similar results had been reported earlier (1990) by the same author. Thus, Ruszkowski (1991) postulated that a monotype plant architecture was essential to increase the yield potential in buckwheat.

5.3.3. Causes of Low Seed Yield in Buckwheat

The yield of buckwheat has shown little improvement in comparison to other crop species. Several authors have suggested different reasons for low yield achievement in buckwheat that have been described below:

5.3.3.1. Self Incompatibility

Pollination and subsequent fertilization is complicated

in buckwheat because of its heteromorphic, sporophytic self-incompatibility system. There are reports that locating bee hives around the buckwheat field increased the yield substantially (Kreft, 1983; Namai, 1990) indicating inadequate pollination and fertilization may limit yield. Pollination may be improved by developing homomorphic lines as proposed by Kreft (1983). However, severe inbreeding depression in such lines precludes their use directly in commercial production (Marshall, 1969).

5.3.3.2. Distribution of Photosynthate

The main factors limiting buckwheat yield were found to be profuse branching and insufficient assimilates for the developing grains (Kreft, 1986; Ruszkowski, 1991). Sugawara (1960) observed that starch, which had very low rate of digestion and translocation, was accumulated in the buckwheat stem. He indicated that translocation of the starch to the developing ovaries may, therefore, be the limiting factor for seed setting. A competition for nutrients has been reported within inflorescences, between inflorescences and between main stem and branches (Ruszkowski, 1990). Higher productivity of non-branching plants in comparison to branching ones has been attributed to the decrease in competition between the main stem and branches (Ruszkowski, 1991).

Kreft (1986) reported that a relatively large sink size as a result of profuse flowering causes shortage in photosynthates. This was indicated by the development of sterile flowers, abortion of developing seeds and the formation of under developed seed, which ultimately resulted in low grain yield and groat or flour recovery. Seed set of less than 12 per cent has been reported in buckwheat. Under stress conditions, the assimilate is translocated only to the vegetative parts resulting in massive death of ovaries and immature seeds (Fesenko, 1990).

5.3.3.3. Indeterminate Growth Habit

The apical meristem was found to be the major sink for photosynthates in buckwheat. An increase in grain yield has been observed upon the removal of new flowering buds, (Kreft, 1983). Therefore, development of determinate cultivars with fewer branches may allow translocation of carbohydrates for the developing grains rather than for the formation of new flower buds that may be too late to develop into seeds. A pleiotropic effect of determinate growth habit was observed in buckwheat genotypes (Luthar, 1986; Kreft, 1989). The determinate plant type had early and uniform ripening of kernels, less shattering of grains, a higher number of kernels per inflorescence and reduced lodging. There was cessation of further growth of the apex and the development of new leaves

and inflorescence. Plant development, flowering and ripening were found to be less sensitive to photoperiod. The only undesirable character was the intense branching that occurred after cessation of apex growth, which could possibly be controlled by high density planting (Ruszkowski, 1990).

In general, the first flowers produced do not develop into seeds. This was described as 'unproductive blossoming' by Kreft (1986). At this period, there was intensive vegetative growth leading to profuse branching, development of new buds and flowers that served as the primary sink for the photosynthate. Therefore, shortening of the unproductive blossoming period by lengthening the productive blossoming may improve grain yield in buckwheat.

5.4. MATERIALS AND METHODS

Ten diverse germplasms originally obtained from various parts of the world were selected for the experiment (Table 5.1). The diverse genotypes were selected to obtain as wide a range of variability in the different traits as possible. The experiment was conducted at the Agriculture Canada Research Station, Morden in the summer months of 1990 and 1991. In both years, the experimental fields had been seeded with wheat in the previous year. The experiment was sown in a randomized complete block design with four replications in

both years. Each plot was made up of four two m rows with a 30 cm spacing between rows. Approximately 30 viable seeds were planted per meter of row length using a mechanical plot seeder. The seeding rate was adjusted for each entry based on seed size and germination percentage.

Germination problems due to a heavy rain, soon after seeding occurred in 1990 necessitating replanting the experiment three times. The last successful planting was done on June 26, which was relatively late. However, timely seeding on June 6th was possible in 1991.

No fertilizer was added to the soil in either years. Plots were hand weeded as necessary. No chemical herbicides or fungicides were applied. Although June was a relatively wet year in 1990, the later part of the season was dry. Therefore, two irrigations were applied on July 24th and August 8th. There was no acute shortage of moisture in 1991 and therefore no additional moisture was supplied. Although no insect or disease problems were observed in either year, the crop was severely hit at the flowering stage by a hail storm in 1991.

After emergence, the seedlings were thinned to a population of approximately 25 seedlings per meter row. Observations were taken on days to seedling emergence, days to

budding, days to first flower, days to 25 per cent flowering, days to 100 per cent flowering, days to first seed setting and days to maturity from the date of seeding. Days to budding, days to first flower and days to first seed set were recorded as the date of appearance of the first flower bud, flower and visible green seed in the plot, respectively. Similarly, days to 25 per cent and 100 per cent flowering were recorded when 25 per cent and 100 per cent of the plants in the plot had at least one flower, respectively.

Due to the diverse germplasm in the experiment and the short growing period, most of the entries did not reach full maturity before the occurrence of the first fall frost. This necessitated recording maturity in terms of the percentage of seed that had turned brown. When the crop approached maturity, twenty plants were randomly marked in each plot and observations on the number of primary branches, the number of axillary flower clusters (inflorescences), the number of terminal inflorescences and plant height were recorded. The mean of all 20 plants was used in analyses of the data. The total plant population per plot at harvest time was also recorded.

After occurrence of the first fall frost, whole plot was swathed on October 10 in 1990 and on September 27 in 1991. The crop was allowed to air dry in the field for approximately

Table 5.1. List of Various Buckwheat Germplasms Utilized to Study the Relationships Among Grain Yield, Yield Components and Various Yield Related Agronomic Traits Grown at Morden, Manitoba in 1990 and 1991.

S. No.	Entry Name	Country of Origin
1.	B640018	Czechoslovakia
2.	B720062	Taiwan
3.	B710065	Poland
4.	B680107	Canada
5.	B730116	USSR (Far East)
6.	B730125	Japan
7.	B740162	USSR
8.	B740169	Canada
9.	B840224	France
10.	B840228	China (Jilin)

one week. Plots were then individually combined. The grains were air dried to 15 per cent moisture and observations were then taken on total grain yield, 1000 seed weight and test weight. Seed yield was converted into g m^{-2} by dividing the total yield by area of the plot.

Based on the above observations, the total number of flower cluster or inflorescences per plant, the number of seeds per plant, the number of seeds per main branch and the number of seeds per inflorescences were calculated. The

number of seeds per plant was determined by dividing the total seed yield by the total plant population at harvest time and dividing by the weight per seed. The total number of inflorescences was calculated by summing the number of terminal inflorescences plus the axillary inflorescences. Similarly, the number of seeds per main branch, and the number of seeds per inflorescence were determined by dividing the number of seeds per plant by the number of main branches and by the number of inflorescences, respectively.

Analysis of variance was performed for each variable to determine differences between genotypes. Homogeneity of error variance for each trait was examined as outlined by Gomez and Gomez (1984) and a combined analysis of variance over years was performed wherever applicable. Significant differences between genotypes were determined by Duncan's multiple range test. Simple correlations among grain yield, yield components and other agronomic characters were calculated using Pearson correlation coefficients.

5.5. RESULTS AND DISCUSSION

Significant differences were found between genotypes in most of the traits observed. Significant differences in error variances were found for grain yield, plants per m^2, seeds per plant, and seeds per flower cluster that did not allow pooling

of the data over years. Therefore, a separate analysis of variance was performed for each year for these traits. Significant differences between genotypes as determined by Duncan's multiple range test are presented in Tables 5A.1 to 5A.16 in the appendix. Simple correlations among yield, yield components and other important agronomic characteristics have been presented in Tables 5.2 to 5.4.

5.5.1. Days to 25 Per Cent Flowering

Highly significant differences between the genotypes were observed for days to 25 per cent flowering. B730125 took the longest time to reach 25 per cent flowering with a mean of 37.5 days, whereas B740169 took the shortest time with a mean of 28 days (Table 5A.3). However, the latter was found to be not significantly different from B710065 and B740162. Observations taken on days to budding, first flowering and 100 per cent flowering after seeding were found to have a similar trend as that of 25 per cent flowering (Tables 5A.1 to 5A.4).

5.5.2. Days to Maturity

Most of the entries did not mature before the occurrence of the first fall frost. Therefore, maturity was taken as the percentage of seed turned brown. A wide range of maturity period was found among the genotypes tested. Genotypes that

flowered early were found to mature early and those flowered late matured late. Lines B730125 and B840224 were found to have the longest maturity period, whereas B740162 had the shortest. However, the earliest maturing genotype was not significantly different in maturity from 50 per cent of the genotypes included in the test (Table 5A.6).

5.5.3. Plant Height

Highly significant differences in plant stature between the genotypes were observed. The late maturing genotypes B730125 and B840224 had the tallest stature with a mean of 1.25 m, whereas the early maturing lines B740169 and B740162 had the shortest stature with a mean of 1.0 and 1.04 m, respectively (Table 5A.7). A highly significant interaction between environment and genotypes indicated that plant height was subject to environmental influence.

5.5.4. Number of Primary Branches

The highest number of primary branches were produced by B730125 followed by B720062 with means of 5.53 and 4.96, respectively. Several genotypes were found to produce branching in the range of 3.6 to 4.0 (Table 5A.8). In general, early maturing genotypes were found to produce fewer branches than late maturing ones, with the exception of

B840224 which, despite its late maturity period, fell into the low branching group.

5.5.5. Number of Terminal Inflorescences

The number of terminal inflorescences, really a measure of the total number of branches, was also found to be significantly different between genotypes. Genotypes producing a high number of primary branches also produced a high number of terminal inflorescences and vice versa (Table 5A.9). This was also true for the total number of inflorescences per plant (Table 5A.10).

5.5.6. 1000 Seed Weight

The mean weight per 1000 seed ranged from 21.5 g to 29.6 g among entries indicating a wide range of variation in this trait. The line B640018 had the lowest seed weight, whereas the line B680107 had the largest seed weight (Table 5A.11). The lines B840228 and B730125, however, did not differ significantly from the line having the largest seed weight B680107. Despite the longer period of maturity taken by B840224, it produced small seed suggesting that late maturing genotypes do not necessarily produce large seed.

5.5.7. Test Weight

Highly significant differences were observed between test weights of various genotypes. A genotype producing the highest seed weight had the lowest test weight and vice versa (Table 5A.12), which indicated that these two traits were negatively correlated.

5.5.8 Plant Stand Per m^2

Although an attempt was made to maintain the same plant population in each plot, highly significant differences for plant stand was observed between genotypes in both years (Table 5A.13). It was clear that genotypes having a higher plant stand also had a higher seed yield, in both years, suggesting that plant stand had a direct relationship on grain yield.

5.5.9. Seeds Per Plant

No significant differences were observed on seeds per plant between the genotypes in 1990, however, highly significant differences were found between genotypes in 1991 that had from 43 to 132 seeds per plant. The lowest number of seeds per plant was produced by the line B730125, whereas the highest number of seeds per plant was produced by B710065

(Table 5A.14).

5.5.10 Seeds Per Inflorescence

No significant differences in seeds per inflorescence was found between genotypes in 1990, whereas highly significant differences were found in 1991 (Table 5A.15). In 1991, the genotype with the highest seeds per inflorescence was B680107 and the lowest was B730125. These were also the highest and the lowest yield producers, respectively.

5.5.11. Grain Yield

In both years, highly significant differences were found for grain yield between the genotypes. Lines B680107 consistently produced the highest yield, whereas B730125 yielded the lowest (Table 5A.16). However, B680107 did not significantly outyield the lines B730116, B740169 and B740162 in 1991. It was found to outyield B730116, B640018, B840228 and B730125 in 1990. Line B730116 was found to be included in the lowest yielding group in 1990, but was among the highest yielders in 1991. Genotypes having a medium maturity period were found to produce the best yields, while the late maturing ones produced the lowest yields.

Due to the occurrence of an early frost in 1991 and late

seeding in 1990, the late maturing lines had a shorter period for seed development when compared to the medium and early maturing lines. Thus, they could not express their yielding ability in either year that could be the probable reason for the observed low yields in late maturing genotypes.

5.5.12. Relationships of Yield and Yield Components

Based on the correlations in Tables 5.2 to 5.4, it was found that some of the yield components affecting yield were consistent over years. Plant stand m^{-2} was highly correlated to grain yield ($r = 0.60^{**}$, 0.67^{**}) in both years. Likewise, seeds per inflorescence ($r = 0.39^{*}$, 0.69^{**}) and seeds per plant ($r = 0.41^{**}$, 0.85^{**}) maintained positive correlations to grain yield in both years indicating that grain yield may have been limited by plant stand. Similar results were found by Bulman and Hunt (1988) in winter wheat, where they observed a positive linear relationship between grain yield and plant stand.

Significant differences on plant stand m^{-2}, seeds per inflorescence and seeds per plant were found in 1991. However, the last two traits were not found to be significantly different in 1990. This suggests that yield differences between genotypes were mainly due to differences in grains per plant and plant stand in 1991, and plant stand

in 1990. Anderson (1986) reported that plant stand in wheat was positively related to grain yield only up to the density of 600 culms m^{-2}. He noted that the response of plant density to yield was highly influenced by season. In this study, plant stand was found to be related to grain yield in both years.

A higher number of seeds per inflorescence and seeds per plant were produced in 1990 as compared to 1991 (Tables 5A.16 and 5A.14). Therefore, the higher yield in 1990 can be attributed to a larger number of seeds being produced. Buckwheat has been reported to be very sensitive to moisture, especially, during flowering period due to its shallow and poor root development (Krotov, 1963; Fesenko, 1990). Therefore, the additional moisture supplied by the two irrigations given during the flowering period in 1990 could have created more conducive environment for grain development as compared to 1991.

Although the number of inflorescences per plant did not exhibit a strong relationship to grain yield in both years (r = -0.19, 0.06), the former was highly negatively correlated to seeds per inflorescence (r = -0.67**, -0.47**). The number of inflorescences, thus, had a negative effect on the grain yield due to competition between developing seeds and flower buds for a limited supply of resources. A negative correlation

between the two traits was found to arise due to intra-plant competition for limited nutrients and metabolites (Westermann and Crothers, 1977).

The number of primary branches was found to be correlated to the number of inflorescences in both years ($r = 0.48^{**}$). This indicated that a high degree of branching, resulting in many inflorescences would not be a beneficial trait in buckwheat. Grain yield in buckwheat, therefore, cannot be improved by simply increasing the number of flowers. Similar views were expressed by Kreft (1983) and Ruszkowski (1990). Kreft (1986) also reported that increased branching in buckwheat resulted in the utilization of most of the nutrients for vegetative growth and only a small amount was available for the developing grains. Similar results were also obtained by Fesenko and Martynenko (1984) where they observed a decrease in yield with an increase in the number of branches.

It has been reported that insufficient photosynthate being supplied to the developing grains was one of the causes of low seed yield in buckwheat (Kreft, 1986; Fesenko, 1990; Ruszkowski, 1991). A relatively large sink size as a result of profuse branching and flowering would result in the diversion of carbohydrate to the floral parts rather than to the developing seeds. Ruszkowski (1989) has reported a high competition between the main stem and the branches for the

Table 5.2 Simple Correlations Among Grain Yield Components and Related Agronomic Traits of Buckwheat Germplasm Grown at Morden, Manitoba in 1990.

YD	MT	FL	GF	SPP	IFP	SPI	TW	SW	PPM
YD	0.22	-0.37*	0.36*	0.41**	-0.19	0.39*	-0.33	0.20	0.60**
MT		-0.84**	0.84**	-0.23	-0.04	-0.23	0.04	-0.02	0.46**
FL			-1.00**	-0.03	0.10	0.02	-0.18	0.15	-0.45**
GF				0.03	-0.11	-0.02	0.18	-0.15	0.45**
SPP					0.07	0.65**	-0.17	-0.12	-0.37*
IPP						-0.67**	-0.29	0.29	-0.31*
SPI							-0.13	-0.29	-0.10
TW								-0.46**	-0.16
SW									0.004
PPM									1.00

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

YD = Yield

MT = Days to Maturity

FL = Days to Flowering

GF = Duration from Flowering to Maturity

SPP = Seeds Per Plant

IFP = Inflorescences Per Plant

SPI = Seeds Per Inflorescence

TW = Test weight (Kg/hectolitre)

SW = 1000 Seed Weight

PPM = Plants Per m^2

Table 5.3 Simple Correlations Among Grain Yield Components and Related Agronomic Traits of Buckwheat Germplasm Grown at Morden, Manitoba in 1991.

YD	MT	FL	GF	SPP	IFP	SPI	TW	SW	PPM
YD	0.38*	-0.55**	0.55**	0.85**	0.06	0.69**	-0.16	-0.07	0.67**
MT		-0.80**	0.80**	0.52**	0.03	0.44*	0.06	0.30	-0.02
FL			-1.00**	-0.68**	0.03	-0.62**	-0.30	0.40**	-0.26
GF				0.68**	-0.03	0.62**	0.30	-0.40**	0.26
SPP					0.11	0.79**	-0.01	-0.46**	0.31
TFC						-0.47**	-0.29	-0.01	-0.13
SFC							0.12	-0.33	0.27
TW								-0.44**	-0.09
SW									0.17
PPM									1.00

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

YD = Yield

MT = Days to Maturity

FL = Days to Flowering

GF = Duration from Flowering to Maturity

SPP = Seeds Per Plant

IFP = Inflorescences Per Plant

SPI = Seeds Per Inflorescence

TW = Test weight (Kg/hectolitre)

SW = 1000 Seed Weight

PPM = Plants Per m^2

Table 5.4 Pooled Simple Correlations Among Grain Yield Components and Related Agronomic Traits of Buckwheat Germplasm Grown at Morden, Manitoba in 1990 and 1991.

	Days to Flowering (DF)	Maturity	Seed Weight	Test Weight	Main Branch	Inflore-scence
DF	1.0	0.82**	0.15	-0.06	-0.10	-0.01
Maturity			-0.02	-0.16	0.21	0.10
Seed Weight				-0.45**	0.29	0.10
Test Weight					-0.46**	0.31**
Main Branch						0.48**
Inflorescence						1.00

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

utilization of the hormone responsible for blooming and seed set in buckwheat. An increased number of branches resulted in a decreased number of seeds per plant in all plant densities observed ranging from 100 to 600 plants per m^2.

Positive correlations between the number of grains per plant with the number of inflorescences ($r = 0.71$) and the number of grains per inflorescence ($r = 0.60$) were reported by Petelina (1980) in buckwheat. However, the present study did not reveal any such relationship between grains per plant and the number of inflorescences ($r = 0.07, 0.11$) in both years. Nevertheless, the relationship between the number of grains per plant and the number of grains per inflorescence were found to be similar to his findings.

The mean 1000 seed weight was found to be not correlated to grain yield in buckwheat in both years ($r = 0.20, -0.07$). It was highly correlated to seeds per plant ($r = -0.40**$) in 1991, but was found to be weakly correlated in 1990 ($r = -0.12$). A weak association in 1990 suggests that there could have been less competition for resources between these traits. A consistent inverse relationship between test weight and grain yield was found in both years ($r = -0.33*, -0.38*$) that suggests difficulty in selecting for large seeded cultivars that produce a high yield. This was further strengthened by the high inverse relationship between test weight and seed

weight (r = -0.44**, -0.46**). Pixley and Frey (1991) have reported a positive correlation between test weight and seed weight in oats.

5.5.12. Relationships of Yield and Phenological Development

Inconsistent relationships between many of the growth parameters were observed over years. A highly significant association (r = 0.40**) between days to flowering and mean seed weight was found in 1991, whereas a very weak relationship (r = 0.15) was found in 1990. The length of pre-blossoming period was inversely correlated to seeds per plant and seeds per inflorescence in 1991 (r = -0.68**, -0.62**), whereas a negligible association was found in 1990 (r = -0.03, 0.02). Similarly, the grain filling period in 1991 was negatively correlated to seed weight (r = -0.40**), while it was positively correlated to seeds per plant (r = 0.68**) and seeds per inflorescence (r = 0.62**). This indicated that plant maturity was restricted and that early flowering genotypes had a longer period for seed development, eventually producing more seeds per plant. The negative relationship between the grain filling period and seed weight can be explained by the positive relationship between the grain filling period and the number of seeds per plant. This indicated a high degree of competition for limited assimilates between the developing achenes. A similar trend was observed

in 1990, but the association was not strong (Table 5.2). Negative relationship between seed weight and seed number have also been reported in cereals by several authors (Fonseca and Patterson, 1968; Campbell and Davidson, 1979; Grafius, 1978; Islam and Langer, 1985).

The percentage of seeds turned brown was found to have no strong relationship with any of the yield parameters in 1990. However, it was found to be significantly correlated with seed yield (r = 0.38*), seed per plant (r = 0.52**) and seed per inflorescence (r = 0.44*) in 1991 (Table 5.19).

The inconsistency of growth parameters between the two years can possibly be attributed to the growing conditions. In general, there was no moisture stress from flowering to maturity in 1990 due to the supply of irrigation water, whereas moisture stress might have occurred during this period in 1991 as no irrigation was given. Similar results have been obtained by Rasmusson and Cannell (1970) in barley where they concluded that phenotypic correlations were highly influenced by the environment and could not be used as a basis for selection for components of yield.

The length of the pre-flowering period was found to be negatively correlated to grain yield in both years (r = -0.36*, -0.55**). This suggested that the early flowering

genotypes had a longer duration from flowering to maturity than did the late flowering ones, which allowed the early flowering genotypes to take advantage of their longer grain filling period resulting in higher yield. Similar results were obtained by Gubbels et al (1990). They observed that early flowering buckwheat genotypes did not suffer yield loss when planted late as did the mid-season genotypes.

A highly significant negative correlation was found between the length of pre-flowering period and the grain filling period ($r = -1.00$). This suggests that an increase in the duration of pre-flowering period resulted in a decrease in the grain development period and vice versa. This also implies that the time to maturity for buckwheat is fixed under Manitoba conditions. This is undoubtedly true that due to late season drought and the occurrence of frost in the early fall, the length of the growing period is restricted. Similar conclusions were reached by Knott and Gebeyehou (1987) and Doguid (1990) in durum wheat and bread wheat, respectively.

A positive correlation between the seed development period and seed yield suggests that improvement in yield can be achieved by a shortening of the pre-flowering period and lengthening the period from flowering to maturity. It appears that an increase in the duration of the flowering to maturity period is possible only by a decrease in the pre-flowering

period taken that the growing period is fixed. Similar results were obtained by Kreft (1983) and Gorina and Anohina (1981 as cited by Kreft, 1983). They found that the length of period from emergence to flowering and flowering to seed maturity were the most important parameters in buckwheat breeding.

Knott and Gebeyehou (1987) reported that if two characters are not correlated, they should be controlled by separate genetic systems. A positive correlation between the two traits suggest that they are controlled by the same genetic systems in the same direction. Since the length of pre-flowering and the grain filling periods were highly inversely correlated, they appear to be controlled by the same genetic system, but in opposite directions. This suggests that it may be difficult to develop early flowering genotypes with long duration from flowering to maturity in buckwheat. The present study, however, does not have enough evidence to fully support this hypothesis.

Unlike cereals, the period from flowering to maturity can not be regarded as the grain filling period in buckwheat. The grain filling period in buckwheat has been reported as 21 to 25 days (Krotov, 1963; Fesenko, 1990), while the duration from flowering to maturity ranges from 60 to 90 days.

5.6. Conclusion

Correlation of yield components and other agronomic traits to grain yield in buckwheat were found to be inconsistent over years indicating they were highly influenced by the environment. However, primary yield components, viz. plant stand, grains per plant and grains per inflorescence had a consistent positive relationship to grain yield. A constant insignificant relationship of seed weight to grain yield suggests that seed weight was not a major factor contributing to yield in this study. Higher yield may be achieved through manipulating plant stand and the number of grains per plant.

A negative relationship between seeds per inflorescence and the number of inflorescence revealed that grain yield in buckwheat was not limited by sink. Therefore, buckwheat yield might be improved by decreasing the number of inflorescences per plant and increasing the number of grains per inflorescence. A highly positive correlation between the number of inflorescences and the number of primary branches could provide an opportunity to reduce the inflorescence number through the development of reduced or non-branching genotypes. An improvement in the yielding ability might also be accomplished by extending the duration from the flowering to maturity period and decreasing the duration of the pre-flowering period.

6. SUMMARY AND CONCLUSIONS

1. A germination medium to assess the viability of buckwheat pollen was developed. Pollen viability as measured by germination percentage in vitro and in vivo was mainly affected by temperature within six hours of the first light. Although a significant effect of temperature was observed at certain periods on the following day, these differences were small and inconsistent. Maximum pollen germination was found two hours and six hours after the first light when the plants were maintained at 25 C and 20 C, respectively. Since most of the buckwheat flowers are pollinated in the morning hours and maximum viability of pollen was found to occur during the same period, production of inviable pollen cannot be considered as a cause of low seed production in buckwheat.

The pollen grains lost viability in less than an hour under room conditions, whereas some pollen grains were found to retain viability for three hours under the same room temperature when high relative humidity was maintained. This suggests that relative humidity was an important factor affecting the pollen longevity.

In this study, pollen viability was tested under two temperature regimes using one genotype. Therefore, further research involving different genotypes and a greater range of

temperature is essential to determine the effect of temperature on pollen viability. The in vitro pollen germination technique developed in this study will be helpful in conducting such research.

2. The extent of natural outcrossing in buckwheat was investigated by utilizing a monogenetically inherited recessive semi-dwarf character. Outcrossing was highest immediately adjacent to the pollen source, but persisted up to 100 m at a lower frequency. There was an incremental decline in the reduction rate as the distance increased, but no detectable difference was found after 9 m from the central plot indicating that pollen is dispersed much farther than 100 m distance. The sharp reduction of pollen dispersal within 3 m distance from the pollen source and lack of directional influence suggested that wind was not a major factor in dispersing the buckwheat pollen. The variation in the frequency of outcrossing throughout the experimental range implies that the intensity of natural outcrossing is not only a function of distance between the two genotypes, but also a function of the foraging behaviour of pollinators. Therefore, the type of insect pollinators in a given locality and their foraging behaviour must be considered in deciding an isolation distance.

Since the degree of outcrossing is also influenced by

spatial arrangement of plots, density of plant population and size and shape of the plots, future research could be focused on these aspects as well.

3. Ten buckwheat germplasms with diverse origin were evaluated over two years at Morden, Manitoba. Highly significant variation between genotypes were found in almost all characters. Primary yield components contributing to buckwheat yield were found to be plant stand, the number of seeds per inflorescence and the number of seeds per plant. Correlation of yield components and other agronomic traits to grain yield were found to be highly influenced by the environment. However, relationships of the primary yield components to grain yield were found to be consistent over years. Based on these relationships, the yield may be improved by selection for increased seeds per plant as seed weight did not show significant effect on grain yield. Since the yield seems to be limited by available photosynthate, a reduction in the number of inflorescences and the number of primary branches through the development of determinate genotypes would allow more assimilate to be available to the developing seeds. The improvement in yielding ability may also be accomplished by selection for a longer duration of the flowering to maturity period through a shorter duration of the pre-flowering period.

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8. APPENDIX

List of Tables in the Appendix.

Table 5A.1 Pooled Data on Days to First Flower Bud From the Date After Seeding (DAS) on Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Number of Days
B730125	28.25 a*
B840224	26.38 b
B720062	26.13 b
B680107	24.13 c
B730116	22.75 cd
B640018	22.50 d
B710065	21.63 de
B840228	21.25 de
B740162	20.75 e
B740169	20.38 e

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.2 Pooled Data on Days to First Flower From DAS on Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Number of Days
B730125	30.13 a*
B840224	30.00 a
B720062	29.63 a
B680107	28.00 b
B730116	27.38 bc
B640018	27.38 bc
B840228	27.25 bcd
B710065	26.88 cde
B740162	26.25 de
B740169	25.88 e

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.3 Pooled Data on Days to Flowering by 25% Plants from DAS on Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Number of Days
B730125	37.50 a*
B720062	34.25 b
B840224	33.75 b
B680107	32.13 c
B640018	30.75 d
B730116	30.50 d
B840228	30.00 d
B710065	28.75 e
B740162	28.00 e
B740169	28.00 e

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.4 Pooled Data on Days to Flower by 100 % Plants from DAS on Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Number of Days
B730125	45.13 a
B720062	40.38 b
B840224	38.25 c
B680107	37.88 c
B730116	35.13 d
B640018	34.13 d
B840228	32.88 e
B710065	32.25 ef
B740162	31.25 f
B740169	31.13 f

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.5 Pooled Data on Days to First Seed Set from DAS on Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Number of Days
B730125	44.25 a*
B840224	42.38 ab
B720062	40.88 b
B680107	38.13 c
B640018	37.88 c
B730116	37.13 cd
B840228	35.75 d
B710065	35.63 d
B740162	35.25 d
B740169	35.25 d

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.6 Pooled Data on Percentage of Seed Browning at the Occurrence of First Frost of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Percentage of Seed Browning
B740162	64.38 a*
B740169	63.75 ab
B840228	58.13 ab
B640018	57.50 ab
B710065	57.50 ab
B730116	55.63 b
B680107	46.88 c
B720062	30.63 d
B840224	26.25 de
B730125	18.63 e

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.7 Pooled Data on Plant Height (cm) of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Plant Height (cm)
B730125	124.88 a*
B840224	124.49 a
B720062	122.33 a
B730116	121.36 a
B680107	113.29 b
B840228	109.71 bc
B640018	106.44 cd
B710065	105.95 cd
B740162	104.21 cd
B740169	100.31 d

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.8 Pooled Data on Number of Primary Branches per Plant of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Number of Primary Branches per Plant
B730125	5.53 a*
B720062	4.96 b
B730116	4.11 c
B640018	4.09 c
B680107	4.08 c
B840228	3.96 cd
B840224	3.79 cd
B740169	3.75 cd
B740162	3.70 cd
B710065	3.61 d

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.9 Pooled data on Number of Terminal Inflorescences per Plant of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Number of Terminal Inflorescences per Plant
B730125	11.60 a*
B730125	11.60 a
B720062	11.28 a
B640018	10.18 ab
B730116	9.44 bc
B680107	9.08 bc
B840228	8.80 bcd
B710065	8.46 bcd
B740162	8.44 bcd
B740169	8.06 cd
B840224	7.14 d

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.10 Pooled Data on the Total Number of Inflorescences per Plant of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotype	Mean Total Number of Inflorescences Per Plant
B720062	45.79 a*
B730125	43.65 ab
B640018	43.26 ab
B730116	41.33 ab
B710065	40.46 ab
B840228	39.96 abc
B680107	38.33 abc
B740162	37.58 abc
B740169	36.46 bc
B840224	32.25 c

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.11 Pooled Data on Weight of 1,000 Grains (g) of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean 1000 Seed Weight (g)
B680107	29.58 a*
B840228	29.26 a
B730125	28.39 a
B730116	25.78 b
B740169	23.85 c
B720062	23.48 c
B840224	23.15 c
B710065	22.98 c
B740162	22.79 c
B640018	21.55 d

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.12 Pooled Data on Test weight (kg/hectolitre) of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Genotypes	Mean Test Weight (Kg/hectolitre)
B840224	65.03 a*
B740169	62.80 b
B640018	62.28 b
B740162	62.03 b
B840228	60.58 c
B710065	59.73 cd
B730116	59.69 cd
B730125	59.25 de
B720062	58.61 e
B680107	57.39 f

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.13 Plant Stand m^{-2} of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Year : 1990 Genotypes	Plant Stand m^{-2}	Year : 1991 Genotypes	Plant Stand m^{-2}
B720062	99.90 a*	B740169	77.22 a
B740169	98.44 a	B840228	75.00 ab
B680107	94.48 a	B740162	73.89 ab
B740162	87.19 ab	B730116	73.06 ab
B840228	86.77 ab	B720062	72.50 ab
B730116	81.88 abc	B680107	71.94 ab
B710065	78.44 abc	B840224	69.31 ab
B640018	71.86 bc	B710065	65.00 bc
B840224	65.21 c	B730125	64.72 bc
B730125	36.25 d	B640018	56.67 c

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.14 Number of Seeds per Plant of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Year: 1990 Genotypes	Seeds/Plant	Year: 1991 Genotypes	Seeds/Plant
B710065	150.37 a*	B710065	132.42 a
B730125	145.41 a	B730116	131.59 a
B740162	140.56 a	B740169	118.36 ab
B840228	124.66 a	B740162	111.75 abc
B680107	123.78 a	B840224	99.30 abc
B720062	122.97 a	B680107	97.21 abc
B840224	116.61 a	B720062	90.86 bc
B730116	111.76 a	B640018	84.60 bc
B740169	102.85 a	B840228	76.75 cd
B640018	101.20 a	B730125	43.38 d

* Means followed by the same letter are not significantly different at 0.05 probability level

Table 5A.15 Number of Seeds per Inflorescence of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Year : 1990 Genotypes	Seeds per Inflorescence	Year : 1991 Genotypes	Seeds per Inflorescence
B840224	4.57 a*	B680107	1.41 a
B710065	3.85 ab	B730116	1.35 a
B740162	3.79 ab	B710065	1.30 a
B680107	3.72 ab	B740169	1.21 ab
B640018	3.23 ab	B740162	1.08 abc
B720062	3.17 ab	B840228	0.98 abc
B840228	3.02 ab	B840224	0.95 abc
B730125	3.01 ab	B720062	0.78 bc
B740169	2.89 ab	B640018	0.62 c
B730116	2.67 b	B730125	0.59 c

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.16 Grain Yield (g m^{-2}) of Buckwheat Genotypes Grown at Morden, Manitoba in 1990 and 1991, Separated By Duncan's Multiple Comparison Test.

Year: 1990 Genotypes	Yield (g m^{-2})	Year: 1991 Genotypes	Yield (g m^{-2})
B680107	333.54 a*	B730116	233.14 a
B840228	315.70 ab	B680107	197.76 ab
B720062	281.86 abc	B740169	191.26 ab
B710065	277.00 abc	B740162	189.24 ab
B740162	257.69 abcd	B710065	181.75 b
B740169	247.36 abcd	B720062	156.47 b
B730116	216.84 bcde	B840228	150.93 b
B640018	214.04 cde	B840224	149.95 b
B840224	175.60 de	B640018	099.67 c
B730125	143.96 e	B730125	077.71 c

* Means followed by the same letter are not significantly different at 0.05 probability level.

Table 5A.17 Monthly Weather Conditions Recorded During 1990 at Morden.

Weather Conditions	June	July	Aug.	Sept.
A. Temperature (C)				
Mean Minimum	12.4	13.9	13.7	9.0
Mean Maximum	17.9	19.6	20.3	15.1
Mean	17.9	19.6	20.3	15.1
B. Precipitation				
Rainfall (mm)	185.4	42.4	47.0	17.0
Days with precip.	15	5	9	2

Table 5A.18 Monthly Weather Conditions Recorded During 1991 at Morden.

Weather Conditions	June	July	Aug.	Sept.
A. Temperature (C)				
Mean Minimum	13.5	14.2	14.2	7.7
Mean Maximum	23.8	25.1	27.8	18.3
Mean	18.7	19.7	21.0	13.0
B. Precipitation				
Rainfall (mm)	171.2	116.8	38.8	77.2*
Days with precip.	14	17	3	12

* Includes snow fall that occurred after the second week.