Inheritance of the Neurotoxin ß-N-Oxalyl-L-α, ß-Diaminopropionic Acid (ODAP) in Grass pea (*Lathyrus sativus* L.) Seeds.

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Inheritance of the Neurotoxin ß-N-Oxalyl-L-α, ß-Diaminopropionic Acid (ODAP) in Grass pea (*Lathyrus sativus* L.) Seeds.

BY

KHUSI RAM TIWARI

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

Master of Science

ABSTRACT

Khusi Ram Tiwari, MSc. The University of Manitoba. Inheritance of the Neurotoxin ß-N-Oxalyl-L-α, ß- Diaminopropionic Acid (ODAP) in Grass pea (*Lathyrus sativus* L.) Seeds. Major Professor, Dr. Clayton G. Campbell.

Grass pea (*Lathyrus sativus* L.) is an important food, feed and fodder legume drought resistant crop. A strong epidemiological association is known to exist between consumption of grass pea and lathyrism. A neurotoxin, ß-N-Oxalyl-L-α,ß-diaminopropionic acid (ODAP) has been identified to be the causative principle. This study was undertaken to investigate the mode of inheritance of the neurotoxin, a prerequisite for the development of neurotoxin free or low neurotoxin lines. Five grass pea lines with low to high ODAP concentration were inter-crossed in all possible combinations (both crosses and reciprocals). Parents, F_1 and F_2 progenies were evaluated under field condition and ODAP analyzed by *ortho*-phthalaldehyde spectrophotometric method. Many of the progenies of low x low ODAP crosses were found to be low in ODAP concentration which indicated that the low ODAP lines shared some genes in common for seed ODAP content. However, wider variation of ODAP in F_2 progenies as compared to parental and F_1 progenies, suggested the presence of different modifier genes between lines. The F_1 progenies of the low ODAP x high ODAP crosses were intermediate in ODAP concentration which indicated lack of

complete dominance either by low or high ODAP. The F_2 progenies segregated covering the entire parental range. The continuous variation, together with very close to normal distribution of the F_2 population both of low x low and low x high ODAP, crosses indicated ODAP to be quantitatively inherited. Reciprocal crosses produced different results in some cases indicating a maternal effect on ODAP concentration. Broad sense heritability of ODAP concentration was estimated to be in the range of 17 to 93%.

DEDICATION

This thesis is dedicated to my parents Mr. Nanda Prasad Tiwari, Mrs. Shanta Tiwari and the victims of lathyrism who have been forced to crawl after the consumption of grass pea seeds as their major staple food.

ACKNOWLEDGEMENTS

I sincerely thank my advisor Dr. Clayton G. Campbell for his successful guidance, valuable time, encouragements and support from the very beginning of this study. I gratefully acknowledge the guidance and support of co-advisor Dr. P.B.E. McVetty and committee member Dr. C. J. Briggs for their time and effort in reviewing this manuscript.

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LIST OF ABBREVIATIONS

BAPN : ß-aminopropionitrile

BOAA : ß-N-oxalylamino-L-alanine

BIA : ß-(isoxazolin-5-on-2-yl)-L-alanine

DABA : α-y-diaminobutyric acid

DAPRO: α,ß-diaminopropionic acid or
2,3-diaminopropionic acid

HCN : Hydrogen cyanide

HCN-p: Hydrogen cyanide potential

HPLC : High pressure liquid chromatography

OAP : L-3-oxalylamino-2-aminopropionic acid

ODAP : ß-N-oxalyl-L-α, ß-diaminopropionic acid or
3-N-oxalyl-L-2, 3-diaminopropionic acid or
3-N-oxalyl-L-2, 3-diaminopropanoic acid

1. INTRODUCTION

Grass pea (*Lathyrus sativus* L.) also called chickling vetch or *Khesari* is an important food, feed and fodder crop belonging to family *Leguminaceae* and tribe *Vicieae* (Smartt, 1990). This tribe is characterized by the possession of leaf tendrils in the majority of its members, an unusual arrangement of vascular tissue in the stem and distinctive floral parts (Goyder, 1986). The genus *Lathyrus* is divided into 13 sections and comprises approximately 150 species (Smartt, 1990), distributed principally over north temperate areas but centred in the eastern Mediterranean region (Goyder, 1986). *Lathyrus odoratus* (Ornamental-Sweet pea), *Lathyrus cicera* (Forage), *Lathyrus sylvestris* (Forage) and *Lathyrus tingitanus* (Forage/Green manure) are other economically important species under the genus and section *Lathyrus*. The word *Lathyrus* is derived from the Greek "Lathuros" meaning a plant, probably a pulse. Possibly it refers to *Lathyrus sativus* itself. Sativus comes from the Latin verb "serere" which means to sow or cultivate, thereby is indicative of that which is cultivated (Westphal, 1974).

Grass pea is a branched, straggling or climbing, herbaceous annual with a well developed tap root system. The stems are normally quadrangular with winged margins. The pinnate leaves consists of one or two pairs of linear-lanceolate leaflets and

a simple or much branched tendril (Kay, 1979). It closely resembles field pea in growth habit but its leaflets are long and grass shaped instead of round and this possibly is the origin of its common name.

Inflorescences are axillary with solitary flowers. The flowers are complete and may be blue, pink, red, purple, tinged or white in colour (Niral *et al.*, 1991). The standard petal is erect and spreading, obovate. Wings are obovate and obtuse at the top. The keel is slightly twisted, boat shaped, entirely split dorsally, ventrally split near the base (Gowda and Kaul, 1982). Stamens are diadelphous (9+1) with a free vexillary stamen. Anthers are ellipsoid and yellow in colour. Ovaries are sessile, widening at tip and bearded below the stigma. Stigmas are terminal, glandular-papillate and spatulate (Gowda and Kaul, 1982). Primarily grass pea is a self-pollinated crop though a significant amount of out crossing (15 to 16%) has been reported under field conditions (Campbell, 1992).

Chromosomal and cytogenetic studies have shown the genus *Lathyrus* to be predominantly diploid with 2n=14 chromosomes. The chromosome number of more than 60 species have been reported with only 3 species having been shown to have more than 14 somatic chromosome. Two species *L. pratensis* and *L. venosus* are tetraploid with 2n=28 chromosomes and one species

L. palustris is hexaploid with 2n=42 chromosomes (Campbell *et al* ., 1994).

Grass pea is cultivated in the Mediterranean area and in the near temperate as well as tropical counties, from the Canary Islands in the west, through Germany in the north, and Ethiopia in the south to India and central Asia in the east (Kislev, 1986). It is an important food and feed legume crop in India, Bangladesh, Pakistan, Nepal, China and Ethiopia. To a lesser extent it is grown in many other countries of Europe (France, Spain), the middle east (Iraq, Iran, Afghanistan, Syria and Lebanon), northern Africa (Egypt, Morocco, Algeria, and Libya) and Brazil and Chile in South America (Campbell *et al.*, 1992). Grass pea ranks first in Bangladesh (Quader *et al.*, 1989), second in Pakistan (Basir, 1989), second in Nepal (Bharati and Neupane, 1989) and third in Madhya Pradesh, India (Lal *et al.*, 1986) among more than a dozen of grain legumes commonly grown in the Indian sub-continent.

The grass pea is endowed with many properties that combine to make it an attractive food crop in drought-stricken, rain fed areas where soil quality is poor and extreme environmental conditions prevail (Palmer, 1989). Despite its tolerance to drought it is not affected by excessive rainfall and can be grown on land subject to flooding (Kaul *et al.*, 1986; Lal *et al.*, 1986; Quader *et al.*, 1987; Rathod, 1989; Campbell *et al.*,

1994). In the Indian subcontinent it is often broadcasted into a standing crop of rice 1 to 2 weeks before the rice is ready to harvest where it flourishes on residual soil moisture left after the rice has been harvested (Bharati, 1986; Lal *et al.*, 1986; Bashir, 1989). Grass pea is often grown as a mixed crop with lentil, linseed, wheat, barley, bengal gram and rapeseed as an insurance against drought so as to have an alternate crop in case of failure of rainfall (Lal *et al.*, 1986; Kaul *et al.*, 1986; Bharati and Neupane, 1989). It is readily cultivated, being typically broadcast sown and flourishes in the absence of tillage and other energy expending cultivation techniques. Normally it is sown in October to November and harvested in March to April in the Indian sub-continent (Lal *et al.*, 1986). It can be grown on a wide range of soil types, including very poor soil and heavy clays as it has a very hardy and penetrating root system. This hardiness together with its ability to fix atmospheric nitrogen makes the crop one that seems designed to grow under adverse conditions (Campbell *et al.*, 1994). Compared to other legumes grass pea is resistance to many pests including storage insects (Palmer *et al.*, 1989). Grass pea has been considered to have potential as a new crop for feed production in the Canadian prairies. A selection and strong breeding program has been instituted at the Agriculture Canada Research Station in Morden, Manitoba. Yields of seed up to the equivalent of 5232 kg/ha have been

obtained in field test plots under good conditions (Briggs *et al.*, 1983).

In the Indian subcontinent two distinct kinds of grass pea are in use (Lal *et al.*, 1986). The small seeded types (5 to 7 g/100 seeds) called *'Lakhodi'* and considered harmless by people are cultivated as *Utera* (relay with rice) where the grass pea seeds are broadcasted into a standing paddy crop, a week to ten days before harvesting the rice. The large seeded types (7 to 15 g/100 seeds) called *'Lakh'* and considered harmful, are generally cultivated as an upland (rainfed) crop.

Grass pea is an important food item of peasants. Most often it is eaten as *Dal* (an aqueous slurry cooked with spices), *Ata* (flour boiled in water) and *Chhatu* (toasted flour mixed with water) (Hamid and Kaul, 1986). It is also commonly eaten as *Chapati* (unleavened bread) or *Dhalpuri* containing varying proportions of wheat. *Bora* is prepared in the form of deep fried paste balls. Khichuri (a Bengali dish) is prepared by cooking rice with variable proportions boiled in water and eaten as porridge. The young plant is taken as a nutritious and leafy vegetable eaten with a rice meal. Grass pea is known to be a survival food during famine (Hussain, 1991). Being relatively cheaper than most other legumes it is also often used as adulterant to chickpea and pigeonpea *Dal* or flour (Gowda and Kaul, 1982; Lal, 1985; Bharati and Neupane, 1989;

Dwivedi, 1989; Deshpande and Campbell, 1992). It is also an excellent fodder and green manure crop (Basir, 1989). After harvesting, the dried straw and chaff are fed to farm animals.

Despite all the advantages as compared to other crops, relatively little effort has been extended to improve this very hardy pulse crop. The main reason has been the knowledge that excessive consumption of grass pea can lead to the neurological disorder called lathyrism (neurolathyrism). This is a neurologic disease characterized by muscular rigidity, weakness, and paralysis of the leg muscle (spastic paraparesis). A water soluble, non-protein amino acid called ODAP (ß-N-oxalyl-L-α, ß-diaminopropionic acid) also known as ß-N-oxalylamino-L-alanine (BOAA) has been identified to be a neurotoxic factor in the seed of grass pea. This has in many cases created the reaction of governments to attempt to ban production or trade of grass pea rather than promoting genetic improvement of the crop (Quader *et al.*, 1989; Campbell, 1989). Indeed, historically, grass pea has been banned by many countries, but it is still produced in significant quantities in many parts of the world (Campbell, 1989).

The urgency of providing neurotoxin free varieties of grass pea to combat lathyrism means that the primary attempt should be to develop very low or neurotoxin free lines. Though the heritability of low neurotoxin concentration seems to be high,

there is no indication of the number of genes involved in the regulation of this character (Gowda and Kaul, 1982). Quader *et al.* (1987) stated that though it is not precisely known how many genes control ODAP content, the available literature suggests that more than one gene may be controlling such a complex quantitative character. The first priority in the improvement of the grass pea is a reduction in the seed ODAP concentration (Smartt *et al.*, 1994). Knowledge of the genetic control of the neurotoxin in grass pea is required for the development of low neurotoxin lines. Hence, this study was undertaken with the following objectives.

1. To study the inheritance of genetic characters affecting the amount of neurotoxin ODAP in grass pea seeds.
2. To determine the genetic relationships, between the three different sources of low ODAP in grass pea seed.
3. To determine the influence of any cytoplasmic factor in seed ODAP concentration.

2. LITERATURE REVIEW

2.1 Origin and domestication of grass pea

Though the origin of grass pea is not known, it is considered to be native of southern Europe and south west Asia (Jackson and Yunus, 1984; Smartt, 1990). Vavilov (1951) described two separate centres of origin of the crop. One was a central Asiatic centre which includes north west India, Afghanistan, the Soviet republic of Tadjikastan and Uzbeskistin and western Tian-shan. The second was the Abyssinian centre. In addition, he also noted that smaller seeded form were found in southern and south west Asia whereas around the mediterranean region almost all were highly cultivated forms with large white seeds and flowers. Jackson and Yunus (1984) also noted that some of the earliest archaeological evidence came from Jarmo in Iraqui Kurdistan, dated at 8000BP[1]. Remains of *Lathyrus* species have been found at Ali Kosh (9500 to 5700BP) and Tape Saby (7500 to 5700BP) in Iran and are amongst the most common foods recorded for these sites. Saraswat (1980) gave a later date of 4000 to 3500BP for the cultivation of *Lathyrus sativus* in India and indicated the possibilities of diffusion of the crop from west Asia. Kislev (1986) suggested that cultivation of *Lathyrus sativus* began in the Balkan Peninsula in the early Neolithic period, around 6000 B.C.E., as a result of expansion of near

[1]Before present.

eastern agriculture. He also noted that *Lathyrus sativus* may have been the first crop domesticated in Europe.

Smartt (1981, 1984a) reported no morphological discontinuity apparent between wild and cultivated *Lathyrus sativus* as compared to species of *Phaseolus* and *Vigna*, possibly due to its use predominantly for forage and less as a pulse. The selection pressure imposed on forage crops are in many ways the opposite of those in grain crops. Development of forms with luxuriant vegetative growth and leafiness may have resulted from selection of forage types (Campbell *et al.*, 1994). *Lathyrus sativus* shows great morphological variation, especially in vegetative characters such as leaf length, while floral characters are much less variable. This array of variation is undoubtly the result of geographical separation as well as selection by man (Jackson and Yunus, 1984). Perrino (1988) reported that some crops, such as wheat and maize, are highly domesticated and are suffering genetic erosion while crops like grass pea are still waiting for further domestication.

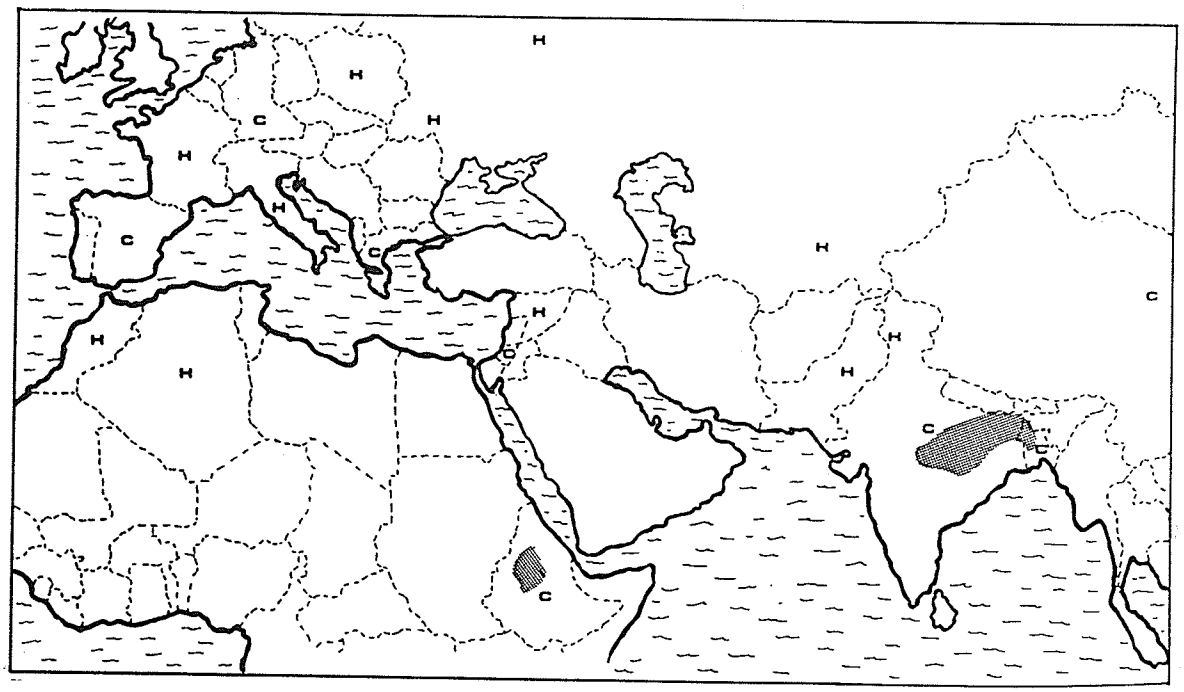
2.2 Lathyrism

The term lathyrism was coined by Cantani in 1873 and describes symptoms following the ingestion of *Lathyrus* species (Cohn and Streifler, 1983). Two clearly different toxic syndromes are known: a) Osteolathyrism; for the effects seen in different

animals following the ingestion of the seeds of *Lathyrus odoratus*, *L. hirsutas* and *L. pusillus* with ß-aminopropionitrile (BAPN) a responsible agent. The pathological changes were mainly in the skeleton and connective tissues b) Neurolathyrism; for the sequela observed in man after consumption of *Lathyrus sativus*. Similar symptoms to neurolathyrism have also been reported in the disease called *Konzo* which is associated with the consumption of unprocessed bitter cassava (*Manihot esculenta*) roots containing high concentration of cyanide (Spencer *et al.*, 1986; Howlett *et al.*, 1990; Tylleskar *et al.*, 1992). Cassava is a perennial, drought resistant, vegetatively propagated shrub grown throughout the lowland tropics for its starchy, thickened roots (Cock, 1982).

2.2.1 History of lathyrism

Among those who knew of the disease were the ancient Hindus, Hippocrates (460 to 377 B.C.), Pliny the elder (23 to 79 A. D.), Galen (130 to 210 A.D.) and the Greek pharmacologist and physician Pedanius Dioskurides (50 A.D.). Duke George of Wurtemburg banned consumption of grass pea flour in his principality in 1671 because of its "paralysing effects on the legs" (Spencer and Schaumburg, 1983). Throughout the 18th, 19th and 20th centuries out breaks of lathyrism have occurred in Europe, northern Africa, the Middle east, Afghanistan, Russia and India (Stockman, 1929; Selye, 1957; Barrow *et al.*,



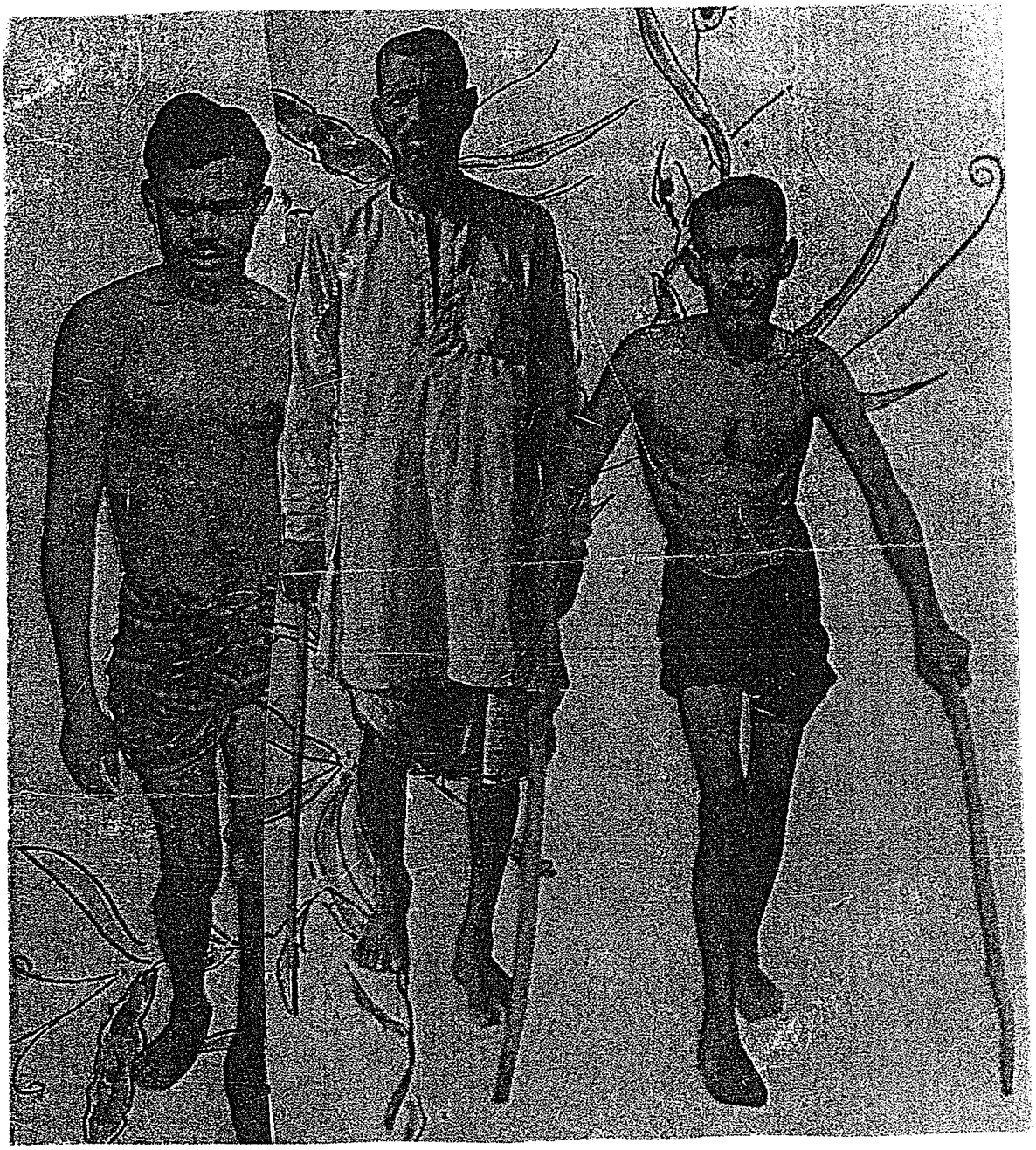
After Spencer *et al.* (1986)

Figure 2.2.1 Distribution of lathyrism: Historical reports (H), Historical and living cases (C) and Actively endemic zones (Shaded areas)

1974; Griffin *et al.*, 1978). Major epidemics have been reported in regions of France (1700 to 1701, 1820s), Algeria (1833), Russia (1892) India (1829 and 1856) and Spain (1940s) (Desparanches, 1829; Proust, 1883; Sleeman, 1884; Semidalov, 1893; Grandjean, 1895 and Stockman, 1917). Lathyrism has been reported from many countries of Europe, Africa and Asia (Figure 2.2.1). During World War II a most remarkable and well documented outbreak of lathyrism occurred among Rumanian Jews confined to a labour camp in the Ukraine. From September 1942, their daily food rations consisted of 400 gm of grass pea seeds cooked in salt water plus 200 gm of bread. In December 1942, a monophasic outbreak of spastic paraparesis involving 800 inmates began mostly among the malnourished. Some victims were unable to maintain bladder function and urinated 30 to 40 times during the night. On January 1943, grass pea was removed from diet preparation. No additional cases appeared afterwards (Spencer and Schaumberg, 1983)

2.2.2 Neurolathyrism

Neurolathyrism is a non-progressive upper-motorneuron degenerative disease similar to primary lateral sclerosis in the chronic stages and usually without involvement of other tissues (Spencer and Schaumburg, 1983). Neurolathyrism appears when *Lathyrus sativus* forms the main item of the diet contributing to at least 30 % of the calories intake for a period of 3 to 4 months (Campbell *et al.*, 1992). Higher level



After Hamid and Kaul (1986).

Figure 2.2.2 Patients of lathyrism with non-stick, one stick and two stick stage of severity in Bangladesh.

of consumption can produce symptoms in only 20 days (Rutter and Persy, 1984; Dwivedi, 1989). Onset is sudden and the victim may suddenly feel weak and the legs have to be dragged with increased effort giving impaired ability to walk (Figure 2.2.2). Onset of paralysis in human may be acute, sub-acute or insidious and is probably contingent on the amount of grass pea consumed and its content of neurotoxin (Hamid and Kaul, 1986). According to the severity of the disease, cases of lathyrism have been divided in 4 stages a) Non-stick stage b) One stick stage c) Two stick stage and d) Crawler stage. Individuals of both sexes and all ages can be affected, but the disease is most prominent among young male adults than females, ratio ranging from 5:1 to 10:1 male to female (Ganapathy and Dwivedi, 1961; Das *et al.*, 1974). The onset of lathyrism in females was either before puberty or after menopause (Dwivedi, 1989) which indicates that the female hormone oestrogen may play an important role in female resistance to the disease (Ganapathy and Dwivedi, 1961).

Lathyrism was found to mainly afflict poverty stricken and underprivileged classes of people. It was most common among those who did not own land or owned very little (Sarker, 1982; Masum, 1982; Dwivedi, 1989). Dwivedi (1989) clearly demonstrated that in the social classes I (richest) to V (poorest), the syndrome did not occur in class I and II and in a season of high incidence (1981) increased rapidly through

social class III (5.65%) and IV (26.5%) to an incidence of 67.8% in one sample of social class V. The practice of giving grass pea seed to agricultural labourers in lieu of wages still exists in the Indian sub-continent (Masum, 1982; Dwevedi, 1989). Ganapathy and Dwivedi (1961) estimated approximately 25000 cases of lathyrism in the Rewa district alone in Madhya Pradesh, India, though occurrence has been reported to have decreased in recent years. Area under grass pea has not been changed in Madhya Pradesh from 1956 to 1985 even after the government's ban on its cultivation in 1963 (Rathod, 1989). Approximately 10,000 lathyrism patients have been estimated in Bangladesh (Kaul *et al.*, 1986). Highest incidence occurred in 1974 when there was a wide spread famine in Bangladesh (Haque and Mannan, 1989). In Ethiopia, though the disease was known for very many years, an epidemic outbreak occurred in 1976 to 77 following drought and flood in the plains of Dembiya and Fogera (Haimanot, 1989). Acharya and Pathak (1990) reported 8 cases of lathyrism in a pilot survey of a few villages in eastern Nepal. In Pakistan, incidence of human lathyrism has been indicated, however, the disease was quite common among the milch buffalo fed extensively on grass pea seed (Basir, 1989).

In a survey performed jointly by a voluntary organization, Shaw Unnayan Sanjstha and Rajasthan University, Bangladesh, revealed that most of the lathyrism affected persons were

landless and suffered from malnutrition (Sarkar, 1982; Masum, 1982). Malnutrition seems closely associated with lathyrism, as a well nourished person has not so far been found to be afflicted with this disease. Vitamin C was found to have a protective role in adult guinea pigs and monkeys (Ahmad and Jahan, 1983; Kabir and Ahmad, 1985). Yusuf *et al.* (1993) reported Zn^{++} to have a protective role against ODAP toxicity in day old chicks. When $ZnSO_4$ was given simultaneously with ODAP, the symptoms appeared later with less severity. Ca^{++} and Mn^{++} were also active in protective action against ODAP toxicity but the effects were milder than Zn^{++}. Lambein *et al.* (1994) postulated a role for brain zinc deficiency in the susceptibility for lathyrism.

2.2.3 Lathyrism in animals

In many countries including the USA and Britain, hind limb paralysis has occurred in domestic animals given grass pea as feed and fodder (Stockman, 1917; Sugg *et al.*, 1944; Kingsbury, 1964). The disease has been reported in several species including ducks, goose, hen, peacock, pig, ox, sheep, elephant and horse (Spencer and Schaumburg, 1983). Signs in the horses include paralysis of the hind legs accompanied by dyspnea and roaring, the latter due to involvement of the recurrent laryngeal nerve. This suggests the equine disease may be more extensive than the human disease, with involvement of the long peripheral nerves as well as the spinal cord and brainstem

(Spencer and Schaumberg, 1983). Chowdhury (1989) reported that grass pea being a low cost protein source, could with advantage be utilized to a greater extent in poultry diets provided safe inclusion levels. Briggs *et al.* (1993) from a feeding study using grass pea seeds as feedstuff in feeder pigs concluded that grass pea meal could be an alternative to other pulses in pig feed, with inclusion rate limited to 10 to 20% to minimize adverse effects. They found that increasing the grass pea content of feed significantly reduced daily weight gain, but pelleting resulted in higher growth rates compared with non-pelleted mass. Diet did not affect carcass grade and ODAP residue were not detected. Meat quality was mainly unaffected. Neurotoxicity was not observed and there was no correlation between ODAP levels and performance.

2.3 Proximate composition of grass pea seed

Grass pea is a versatile and highly palatable component of human food and is a good animal fodder. The food quality is good, protein content is high (28 to 30%) and amino acid composition fairly balanced. The fat content is low and the vitamin content is good, except for a paucity of vitamin A and C (Palmer, 1989). Analysis of the whole seeds of grass pea have revealed the proximate composition to be: dry matter 87 to 90 %; crude protein 24 to 30 %; ether extract 0.6 to 5.2 %; ash 2.7 to 3.3 %; crude fibre 5 to 8.8 % and carbohydrates 46 to 58 % (Rudra, 1952; Sarma and Padmanaban, 1969; Latif *et al.*

1975). The amino acid analysis of the seeds of grass pea after acid hydrolysis were performed by Sarma and Padmanaban (1969) and Latif *et al.* (1975). Their results showed that the seed is rich in lysine and contains relatively adequate concentrations of other essential amino acids except cystine and methionine. Tryptophan content of different varieties ranged from 0.09 to 0.23% on a dry weight basis. These differences in tryptophan content may be attributed to cultivar and/or environmental variations.

2.4 Non protein amino acids

Out of approximately 700 non-protein amino acids (i.e. amino acids that are not found as building blocks of protein) known to date in the plant as well as animal kingdom, the tribe *Vicieae* comprising *Vicia, Pisum, Lathyrus, Lens*, and *Vavilovia* has been the source of around 42 of them (Lambein *et al.*, 1990). Some of these amino acids have received a substantial amount of interest considering their physiological effect on man and animals. The neurotoxic principal ODAP of grass pea is in this class of compounds. Since its isolation and characterization approximately 30 years ago, various research groups have been involved in its chemical and biological synthesis, isolation, physical and chemical properties determination, distribution studies in other species, development of methods for analysis, and toxicological studies (Kebede, 1993).

2.4.1 Neurolathyrogen-ODAP

Several factors in the past were believed to cause lathyrism. These included phytates, alkaloids, lack of vitamins A, B, and C, and virus infection (Sarma and Padmanabhan, 1969). High selenium and low methionine in the grass pea seeds were also considered to cause lathyrism. The seeds of *Vicia* species present as contaminants in market samples of grass pea seeds were claimed to cause toxicity (Ressler, 1962). However, the active toxic component from the seeds of grass pea that is considered responsible for human lathyrism has been identified as ß-N-oxalyl-L-α, ß-diaminopropionic acid (ODAP) otherwise designated as ß-N-Oxalylamino-L-alanine (BOAA) or L-3-Oxalylamino-2-aminopropionic acid (OAP) by two groups of investigators independently and concurrently (Murti *et al.*, 1964; Rao *et al.*, 1964). It is a water soluble non-protein amino acid which is ninhydrin positive and gives a purple colour with ninhydrin. Its chemical structure is as follow: $COOH\text{-}CO\text{-}NH\text{-}CH_2\text{-}HCNH_2\text{-}COOH$ (Briggs, 1989).

ODAP has been detected in the seeds of 21 *Lathyrus* species, 17 *Acacia* species and 13 *Crotolaria* species (Quereshi, 1977). On a dry weight basis the concentration of ODAP in the seedlings of grass pea are usually about two to three times higher than that in dry seeds (Lambein *et al.*, 1993). Bell and O'Donovan (1966) observed that the naturally occurring ODAP exists in two isomeric forms, alpha and beta which are

separable by high voltage electrophoresis. The alpha and beta forms both give oxalic acid and alpha beta diaminopropionic acid on hydrolysis. Roy and Rao (1978) confirmed these findings and showed that, irrespective of the concentration of ODAP in different varieties of grass pea, the proportion of alpha and beta isomer of the amino acid remains unchanged. The beta isomer in grass pea ranges from 92% to 96% of the total ODAP content (Roy and Rao, 1978). The alpha isomer is not known to be toxic.

2.4.1.1 Biosynthesis

To develop a cultivar free from neurotoxin, information on the biosynthetic pathway and the enzymes involved in its biosynthesis may eventually become important, if modern technology, including genetic engineering, is to be utilized (Hussain, 1991). Biosynthesis of ODAP had been a challenging problem which has so far defied most approaches used for tracing its biosynthetic pathway. Though the neurotoxin ODAP was identified 30 years ago, until recently its biosynthetic pathway was obscure. Lambein et al (1990) concluded that, in the young seedlings of *Lathyrus sativus* L., ß-(isoxazolin-5-on-2-yl)-L-alanine (BIA) was the precursor for the *in vivo* biosynthesis of the neurotoxin ODAP responsible for human lathyrism, and that the cotyledons were the major site of this biosynthetic step in the seedling stage. The Lambein group (1990) proposed the following biosynthetic pathway for ODAP:

1. Asparagine--------> Isoxazolin-5-one---------->BIA-------DAPRO------Oxalyl CoA => ODAP.

The young leaves and ripening seeds of mature green plants contain high concentrations of ODAP without detectable amounts of BIA present and a different pathway for the biosynthesis of ODAP cannot be excluded (Lambein *et al.*, 1990). Kuo and Lambein (1991) studied the biosynthesis of ODAP in callus tissue of grass pea and confirmed the previous finding that BIA was the precursor for ODAP and indicated that DAPRO (α,ß-diaminopropionic acid) may indeed be the short-lived intermediate in the pathway. BIA was also found in the seedlings of the genera *Pisum*, *Lens* and *Vicia* including *Lathyrus* species (Lambein *et al.*, 1990). BIA is not found in ripe seeds but it can reach a concentration of 2% in the seedlings (Lambein *et al.*, 1990). It is also selectively released by pea seedlings roots. BIA was not detected in seeds of *Crotolaria* and *Acacia*. Kuo *et al.*(1993) studied the biosynthesis of ß-ODAP in *Crotolaria* by incubating ^{14}C BIA by imbibing seeds overnight. No radio-activity was found in purified ß-ODAP suggesting an alternate pathway in *Crotolaria* species.

2.4.1.2 Determination of ODAP

Various methods for the determination of the level of ODAP in grass pea and lathyrism related samples exist. Electrophoresis

and paper chromatography give separation of the two isomers (α and ß) but quantitative work is either unreliable or the time required for screening large accessions impractical (Briggs, 1989). A convenient spectrophotometric method developed by Rao (1978) and modified by Briggs *et al.*(1983) utilizing the reaction of the alkaline hydrolysis product of ODAP, 2,3-diaminopropionic acid (DAPRO) with orthoph-thalaldehyde is presently being utilized at many research laboratories (Kebede, 1993). A quantitative relationship between the concentration of diaminopropionic acid and the intensity of the yellow colour produced when the *ortho*-phthalaldehyde reagent is added to the solution formed the basis of a spectrophotometric assay for free diaminopropionic acid in seed samples (Briggs *et al.*, 1983). In this procedure, hydrolysis of the extract by potassium hydroxide was complete after 30 minutes. However this procedure does not distinguish between the α and ß isomers and pigments can interfere with the analysis to determine the ODAP concentration of the plant part (Briggs, 1989).

Khan *et al.* (1993) presented a method for determining ß-ODAP, the potent neurotoxic substance of the seeds and seedlings of *Lathyrus sativus* L., and α-ODAP (non toxic isomer). The separation of the two forms was achieved after derivatization with phenylisothiocyanate employing a high pressure liquid chromatography (HPLC) system. This method was used to monitor

the isomerization of ß-ODAP to α-ODAP at different time intervals and to quantify the toxin level in seed extracts. Geda *et al.* (1993) also recently reported a sensitive spectrofluorometric HPLC method suitable for determining ODAP in individual seeds and to assay ODAP in animal tissue at submicro-gram levels.

2.4.1.3 Evolutionary significance of ODAP

The physiological role of this neurotoxic amino acid within the plant system is not yet well understood (Kebede, 1993). Plants are known to synthesize and utilize diverse types of compounds for different purposes. Amino acids have key functions in the plant metabolic processes (Stewart and Larher, 1980) besides their role as building blocks of proteins. Non-protein amino acids, toxic to insect and mammal, have been identified from plants. They are considered to be part of the defence mechanisms of some species against predators (Jolivet and Mosse, 1982). On the other hand, stimulation of microorganisms has been achieved by plant exudate leading to beneficial ecological relationships between them (Kebede, 1993).

Roy and Bhat (1975) studied the neurotoxin ODAP variability and the susceptibility to insect (*Callosobruchus chinensis*). They could not find any association between the susceptibility to insect attack and ODAP concentration. On the other hand

there was a significant positive correlation ($P < 0.05$) between trypsin inhibitor content and percent susceptibility to insect attack.

As grass pea is known as a drought tolerant crop, ODAP could be associated with this mechanism. Comparison of the root/shoot fresh weight ratio of high and low neurotoxin varieties showed no correlation between the toxicity and the drought tolerance of grass pea. It is unlikely that the neurotoxin ODAP plays a major role in the drought tolerance of the plant (Ongena *et al.*, 1990). However, recently it was found that α-ODAP facilitates the uptake of Zn^{2+} ions by plants of grass pea grown under Zn-depleted condition, while the enantiomer D-ODAP, which does not occur as a natural product, did not show such physiological effect (Kebede, 1993). Furthermore, the absence of Zn^{2+} in the nutrient solution resulted in higher production of ODAP in the plant, both in the root washing and the seeds. Lambein *et al.* (1994) proposed that the neurotoxin ODAP to be a biologial carrier for the uptake and transport of zinc ions in grass pea.

2.4.1.4 Detoxification of ODAP

To minimize the toxic effect of grass pea in the human diet two possible ways have been approached.

a. Physico-chemical detoxification: The following methods of detoxification have been suggested. These methods remove

approximately 80 to 90% of the neurotoxin (Bell, 1964; Mohan *et al.*, 1966; Sharma and Padmanabhan, 1969; Ahamad and Jahan, 1983; Chimnoyee *et al.*, 1991).

a. Steeping dehusked seed in hot water for several hours.
b. Boiling the seed in water and draining the supernatant.
c. Parboiling.
d. Roasting of seeds at 150° C for 20 minutes.
e. Decortication of ground grass pea seeds soaked in saturated lime water for 2 to 3 hours and autoclaved for 10 minutes at 103.5 K pa.
f. Boiling in lime water for 30 minutes.

These detoxification methods have not been widely adopted possibly because they were not very effective mainly due to loss of water soluble nutrients, reduce weight of grains, reduce taste and the glutenous property of different food preparations. Extra fuel requirement was also a serious handicap along with water scarsity during the periods of drought.

b. Genetic detoxification

Genetic detoxification is the most feasible solution to the neurotoxin problem in grass pea (Smartt *et al.* 1994). This method involves selection and breeding for low neurotoxin lines. Screening of germplasm has resulted in identifying

several lines that have low ODAP concentration which are lower than that obtained by processing (Jeswani *et al.*, 1970; Nerkar, 1972; Misra *et al.*, 1979; Ramanujam *et al.*, 1980). Lines are now available having as low as 0.03% ODAP as compared to 0.3 to 1% commonly grown in the Indian subcontinent (Campbell and Briggs, 1987). Quader *et al.* (1989) reported ODAP concentration of 0.01% in selected F_2 plants. Roy *et al.* (1993) derived somaclones with ODAP concentration of 0.01% on seed and 0.015% on leaf from internode explants of grass pea. There are encouraging precedents in other grain legumes such as lima beans and lupins, where contents of cyanogenic compounds and alkaloids have been reduced to acceptable levels (Smartt *et al.*, 1994). In both lima beans and lupins there are polymorphisms for toxin concentrations in the seed and it was a logical step to explore the possibility of a similar polymorphism in the grass pea.

2.4.2 Other non-protein amino acids

Lambein *et al.* (1992) reported other non-protein amino acids present in grass pea as follows:

Homoserine: This non-protein amino acid is rapidly formed during germination and reaches up to 12 % of the dry weight in pea (*Pisum sativum*) seedlings. It is present in many of the *Vicieae* species including grass pea.

Homoargenine: Is present in seed with concentration decreasing during germination and being absent from older plants. It is a non-toxic amino acid.

Isoxazolinone derivatives: These are heterocyclic amino acids formed with an isoxazolin-5-one ring. These compounds rapidly increase during germination and are virtually absent from dry seed and older plants. Lambein *et al.* (1986) reported prevalence of isoxazolinone compound only in tribe *Vicieae* in the plant kingdom. All species of *Pisum, Lens* and the majority of *Lathyrus* species contained this compound. They reported 36 species of *Lathyrus* containing different combinations of isoxazolinone compounds. In grass pea four major isoxazolinone derivatives were detected (Lambein, 1989):

Compound I: ß-(Isoxazolin-5-on-2-yl)-alanine (BIA)

Compound VI: α-amino-y-(isoxazolin-5-on-2-yl)-butyric acid

Compound VIII: 2-cyanoethyl-isoxazolin-5-on

Compound XI: y-glutamyl derivative of I

Compound VI and VIII are reported to be neurolathyrogenic and osteolathyrogeic respectively hence may increase the general toxicity of grass pea in the seedling stages (Lambein, 1989; Lambein *et al.*, 1992)

Rukmini (1969) isolated a toxic glucoside from grass pea seed known as N-ß-D-glucopyranosyl-N-α-L-arabinosyl-α,ß-

diaminopropionitrile. This toxin is present in very low concentration (0.003%) and a high dose is needed for toxicity, hence, it may not be of any significant importance.

2.5 Genetics of ODAP

2.5.1 Genetic variability of ODAP

Considerable variability has been reported on ODAP concentration among grass pea lines. In an analysis of approximately 3000 samples from indigenous and exotic source at the Indian Agricultural Research Institute (IARI) New Delhi and other institutions in India, ODAP concentration was found to range from 0.1 to 2.5% (Nagargan and Gopalan, 1968; Jeswani *et al.*, 1970). Kaul *et al.*(1982) reported ODAP ranging from 0.61 to 1.18 % with a mean of 0.87 % in a germplasm collection at the Bangladesh Agricultural Research Institute (BARI), Dhaka Bangladesh. Campbell and Briggs (1987) identified and registered a low neurotoxin line of grass pea with a mean ODAP concentration of 0.03% in the seed. Roy *et al.* (1993)reported ODAP concentration of 0.01% in seed and 0.015% in leaf from somaclones derived from internode explants of grass pea. They found a significant positive correlation between leaf and seed ODAP concentration (r=0.766). They further stated that somaclones with extremely low ODAP concentration also had seed yield equal to or higher than parent cultivar P-24, and thus indicated a distinct possibility of cultivars having a low neurotoxin coupled with high yield. Nagarajan *et al.* (1968)

analyzed the straw of grass pea for ODAP concentration and found it to vary from 0.1% to 0.6% on the sample weight basis.

Przybylska and Rymowiej (1965) detected maximum concentration of ODAP in the leaves of the grass pea plant. Prakash *et al.* (1977) reported the distribution of ODAP in developing grass pea plants. They concluded that, irrespective of varieties and stages of plant development, ODAP was present in all tissues, but that its maximum concentration was present in the leaf during the vegetative phase and in the embryo during the reproductive phase. They also noted that young leaves contained higher concentration of the neurotoxin as compared to the older leaves.

2.5.2 Environmental influence on ODAP

Environment is reported to play an important role in the production/synthesis of ODAP in grass pea (Kaul *et al.*, 1986; Campbell, 1989; Bell, 1993). Grain yield have also been reported to be highly influenced by the environmental conditions such as moisture conditions and relative humidity (Swarp and Lal, 1993). Kuo *et al.*(1990) reported that water stress can result in an increase of virtually all amino acids in grass pea including a doubling of the concentration of ODAP. A similar observation was made by Campbell (1990) under field conditions. Hussain *et al.* (1993) from their study made the following conclusions; a) water stress condition in

laboratory pot culture and in the field (drought area) enhanced ODAP concentration in grass pea seed b) the neurotoxin concentration appeared to decrease in seeds grown in the field soil kept saturated with water but the findings were variable c) salinity decreased ODAP concentration in seeds d) zinc deficiency did not affect ODAP concentration and e) foliar application of micronutrients like cobalt and molybdenum and application of fertilizer failed to influence ODAP concentration. Similarly, Haque *et al.* (1993) reported a decreasing concentration of ODAP with increased salinity. However, they reported that excess supplement of Zn had a cumulative decreasing effect on amino acids. Mn, Mo and Mg showed a decreasing effect on the ODAP level but B and Co were found to have no effect. They also reported quite a high tolerance of grass pea to aluminium which had a prominent increasing effect in ODAP concentration. Lambein *et al.* (1994) recently reported that zinc deficiency and over supply of iron to the roots of grass pea increased the concentration of neurotoxin ß-L-ODAP. They also postulated that soils depleted in micronutrients from flooding by monsoon rains (Indian sub-continent) or otherwise poor in available zinc and high iron content (Ethiopian Vertisols) may be responsible for higher incidence of human lathyrism.

2.5.3 Genotype x environment interaction on ODAP

Dahiya and Jeswani (1975) found that the stability parameters for ODAP due to genotype and environment as well as genotype x environment were significant. The genotypes differed significantly in their performance. The environments were also quite variable. Ramanujam *et al.* (1980) reported that ODAP concentration was subject to large genotype x environment interaction. Dahiya (1986) from his experiment of 18 genotypes, 2 locations and 3 fertility levels concluded that, ODAP concentration was highly affected by location effects. He found Pusa-24 to be more stable than others in its response to changing environments. He also noted that different fertility levels did not affect ODAP concentration in the seeds. Kaul *et al.* (1982) on the other hand reported that environment did not have much effect on ODAP. However, their inference was based only on data of 18 lines over two environments offering little scope for expression of genotype x environment interactions.

2.5.4. Association of ODAP with other traits

Misra *et al.* (1979) reported a significant negative correlation of ODAP with protein content in a germplasm collection maintained at the Division of Genetics, IARI, New Delhi. However, in a subsequent detailed study, no association was found to exist between ODAP and protein content, instead a positive correlation between seed weight and ODAP concentration was reported (M. Hussain, Personal

communication, 1984). Dahiya (1976) indicated that ODAP and seed size in grass pea were positively correlated and he noted that light cream coloured seeds from blue flower coloured plants were associated with low ODAP. However, his inference was based on only nine tested genotypes. Quader *et al.* (1986) reported white flower colour plants have more toxin as compared to blue flowered plants. Nagarajan and Gopalan (1968) on the other hand indicated no relationship of seed coat colour with ODAP concentration. Similarly Kaul *et al.* (1982) could not establish any relationship of ODAP with any of the above mentioned characters (protein, seed coat colour, flower colour or seed size).

2.5.5 Biotechnology and ODAP

Genetic engineering has a systematic approach to varietal evolution since it is based on identification, characterization, and transfer of specific genes into the recipient plants as compared to the mixing of two complete genomes of two parental lines followed by back crossing for several generations to remove the undesirable genes (Campbell *et al.*, 1994). This strategy could be successfully exploited to develop lines with low or zero level of ODAP. However, a prerequisite for this is the identification of the source gene which eliminates the ODAP and plant regeneration techniques. Mehta *et al.* (1991) reported a *Pseudomonades* microbe which carries genes for ODAP degradation on its plasmid and utilizes

ODAP as its sole carbon and nitrogen source. Datta (1993) reported that they had isolated and characterized a novel gene, oxalate decarboxylase which could serve as a tool to degrade oxalic acid in plants. Oxalic acid is an essential starting substrate for ODAP synthesis. Plant regeneration techniques both from leaf (Roy *et al.*, 1991) and root (Roy *et al.*, 1992) explants have been reported which will allow its use, not only for application to plant genetic engineering but also for exploitation of somaclonal variation and use in conventional plant breeding. These findings open up the possibilities of developing grass pea lines with zero level of ODAP which will be a significant contribution to exploit the potential of this highly neglected, drought and flood resistant, protein rich crop in the semi arid regions of the world.

2.5.6 ODAP Inheritance

There are very few studies on the mode of inheritance of ODAP. Nerkar (1972, 1990) reported that ODAP content might exhibit a simple mendelian inheritance from variations induced in grass pea through both physical and chemical mutagens. In the segregating M_2 generation in all treatments, the distribution curves showed three distinct peaks, characteristic of monogenic F_2 segregation. However, Dahiya (1986), reported that ODAP is quantitatively inherited. Quader (1985) studied F_2 segregation behaviour of crosses involving parents with

low and high ODAP concentration. He found almost continuous variation with 3 peaks suggesting monogenic control. He postulated that a major gene with a few modifiers might control the synthesis of ODAP. Briggs and Campbell (1990) reported the presence of more than one gene to control ODAP production in line LS82046. They also observed heterosis for ODAP concentration in a cross between high and low or medium ODAP lines. Biosynthetic pathways studies by Malathi *et al.* (1967) and Lambein *et al.* (1990) revealed that at least two enzymes were involved in the synthesis of the neurotoxin. This suggested that probably more than one gene may be controlling ODAP synthesis. Quader *et al.* (1987) reported that more than one gene may be controlling such a complex quantitative trait as ODAP concentration. Thus, the literature concerning the inheritance of the neurotoxin ODAP is very scant and conflicting.

3. MATERIALS AND METHODS

3.1 Green house works and crossing procedure

Of the five lines of grass pea that were included in this study, four were low and one was high in ODAP concentration (Table 3.1). The line LS82046 was derived from Pusa-24 while L900436 had Pusa-24 as one of its parents. Crosses were made in the green house in all possible cross combinations including reciprocals, to produce the 20 possible cross combinations. Pot mix was prepared from 2 parts of soil, 1 part peat moss and 1 part of vermiculite. All the seeds were scarified with a file for uniform and rapid germination. Plants were watered as needed.

Table 3.1 General characteristics of grass pea lines used in this study.

Lines	Origin/Source	ODAP
1. L720060	France/2R-151	Low
2. L900436	Bangladesh/V 8603	Low
3. LS82046	India/L750074/P-24	Low
4. LS90235	France/L740084	Low
5. L880283	Germany/PGR 19761	High

The five parental lines were seeded on January of 1992 to begin the crossing procedure. Plants initiated flowering approximately 42 days from seeding. All lines were uniform in flowering time with the exception of L900436. This line was quite late under green house condition and pollen production was very low, whereas under field condition, flowering was synchronized with other lines. Subsequent attempts were required to make crosses with this particular line by seeding on different dates and growing it in a growth room in a higher temperature regime. Pruning of flower buds and pods of other lines was done to extend the flowering period.

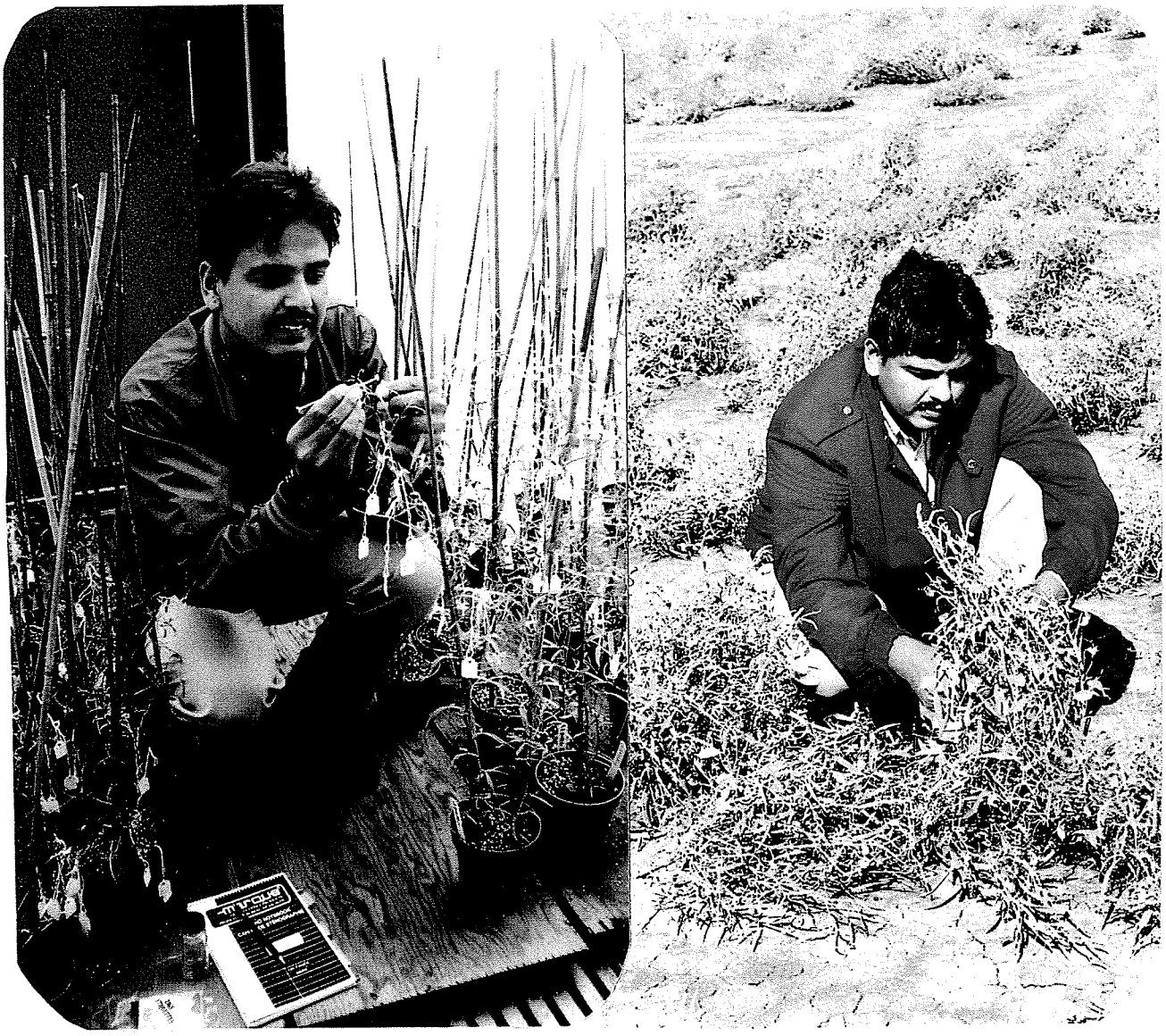
Emasculation of the female flowers was done in the morning hours with the help of forceps. Selection of optimum bud size was very crucial, since smaller buds give a very low success rate and larger buds increase chances of accidental selfing. The forceps were disinfected with 70 % ethanol after each flower emasculation. Pollination was done 12 to 24 hours after emasculation when the stigmas became receptive. Flowers in full bloom were sacrificed and the pollen applied manually on the stigmatic surface of the recipient parent. Pollen of one flower could be used for 1 to 3 pollinations. After pollination, individual flowers were tagged with information on male source, female plant number, date of cross, etc (Figure 3.1). After approximately one week, each crossed flower was examined to determine if pollination had been

successful. Variation on pollen production and success rate was observed both within and between lines. Some lines were good male parents whereas others were good females. Line L900436 was a very good female parent and a poor male parent. The success rate of crosses varied from less than 5 to more than 70%.

Parents, F_1 and F_2 progenies of all low ODAP by low ODAP combinations (six crosses and the reciprocals) were grown in the field in the summer of 1992, whereas progenies of all low ODAP by high ODAP (four crosses and the reciprocals) were grown in the summer of 1993. All parents, F_1 and F_2 progenies were grown in the same field to minimize environmental variability among crosses since ODAP has been shown to be highly influenced by the environmental factors. Flower colour, seed coat colour, 100 seed weight, individual plant yield and ODAP concentration were recorded on all individual plants.

3.2 Field experiments

Trials were grown in a sandy loam soil, at the Agriculture Canada Research Station, Morden, Manitoba, Canada. Soil test results for macro and micro nutrients indicated that all nutrients were in the range of high to very high and thus no fertilizer was applied. A soil test by the Manitoba Provincial Soil Testing Laboratory showed the following analysis from the top 15 cm of soil: Organic matter 5 to 6 %, soil pH 7 to



(a) (b)

Figure 3.1 Grass pea (a) crossing procedure in growth room and (b) evaluation of the progenies under field condition at Morden Research Station, Morden, Manitoba, Canada.

7.3, Nitrate Nitrogen 42 to 53 kg/ha, Phosphorus 40 to 48 kg/ha, Potassium 800 to 1100 kg/ha, Sulphate sulphur 15 to 18 kg/ha, Calcium 7580 kg/ha, Magnesium 1440 kg/ha, Copper 5.7 kg/ha, Iron 107 kg/ha, Manganese 41 kg/ha and Zinc 7.5 kg/ha.

Fall application of Trifluralin (Treflan) at the rate of 3 L/ha in 200 litres of water was applied to suppress weed growth. Occasional manual weeding was required, however, to keep the plots weed free. Aphids (*Aphis craccivora*) were a problem in both years in the field and a mixture of Malathion plus Laigon was applied at 0.2 % concentration. Two applications were required at a 2 week interval. No incidence of disease was recorded under field condition.

Planting was done with a small plot seeder. Scarification, particularly of green house grown seed was necessary to insure uniform germination. A simple scratch with a file was sufficient for water imbibition and rapid germination. Inter-row and intra-row spacing was maintained at 1 m and 0.3 m respectively. Thinning was done to maintain the intra-row spacing two weeks after emergence. The F_2 plot size was 8 rows, 13 m long. Parents and F_1 progenies were grown in 1 row each. A check line, LS90043, was planted in border rows, in both years to evaluate year effect on ODAP concentration. Individual plants were harvested, threshed and dried at 40°C for 3 days to bring the final moisture content to 8 %.

3.3 Chemical Analysis

ODAP concentration was determined spectrophotometrically using a *Ortho*-phthalaldehyde fluorescent dye with minor modifications of the method described by Briggs *et al.* (1983). All samples were analyzed using a fast screening wet chemistry method as follows:

1. Preparation of reagents

a. Potassium hydroxide (3N)

Add 168.33 g KOH per liter distilled water.

b. Potassium borate buffer (self life of one month in a well stoppered bottle).

3.092 g potassium borate

3.728 g potassium chloride

3 pellets sodium hydroxide

999 mls distilled water

pH was adjusted to 9.9 with concentrated sodium hydroxide solution.

c. O-phthalaldehyde reagent (OPT)

1000 µl ethanol (95 %)

100 mg O-phthalaldehyde

200 µl mercaptoethanol

99 ml potassium borate buffer.

2. Extraction procedure

Samples were obtained by grinding hand cleaned seeds in a Wiley Intermediate Mill (20 Mesh).

Powdered seed samples of 0.5 gm were extracted with 10 ml of 60 % ethanol, vortexed and shaken continuously for 45 minutes. Samples were centrifuged at 4500 rpm for 15 minutes.

3. Hydrolysis

Two ml of sample aliquots were then mixed with 4 ml of 3N KOH in screw capped test tubes and hydrolysed in a boiling water bath for 30 minutes.

A blank sample was prepared using 2 ml of 60 % ethanol instead of 2 ml sample aliquot.

Again the tubes were centrifuged at 4500 rpm for 15 minutes

4. Colour reaction

250 µl of aliquots were made to 1 ml with distilled water.

2 ml of *Ortho*-phthalaldehyde reagent was added.

Samples were incubated for 2 hours at 41°C.

Spectophotometric readings were taken at 425 nm by setting absorbance of the blank as zero.

ODAP concentration in the samples was determined from standard graph.

A quantitative relationship between the concentration of diaminopropionic acid and the intensity of the yellow colour produced when the *Ortho*-phthalaldehyde reagent is added to the solution is the basis of this assay. However a orange to red colour was also observed in some samples which could interfere

in the assay and give an erroneously high reading. To overcome this problem duplicate sample aliquots were prepared and potassium borate buffer was added in place of *Ortho*-phthalaldehyde (OPT). This blank would then give a reading of interference due to colours other than the yellow colour produced with the reaction of OPT solution and diaminopropionic acid. Analysis of over 100 samples with the addition of Potassium Borate buffer instead of *Ortho*-phthaldehyde reagent, gave an average reading of 0.02 which indicated the absorbance value other than that of ODAP. Hence, the ODAP concentration was determined in the samples from the standard graph after substracting 0.02 from the sample reading.

A reference sample of grass pea seed with known ODAP concentration was analyzed as an internal standard with each set of samples to ensure accuracy and reproducibility. Single sample analysis for ODAP were deemed adequate, since this method has routinely been used in this laboratory for the past 10 years for approximately 4000 samples annually. During this time frame, duplicate analysis of randomly selected samples have shown a correlation of 0.998.

3.4 Data analysis

Data was subjected to simple statistical analysis and to frequency distribution. Test statistics (t test) was used to

determine the statistical differences between two means (cross and the reciprocal). Broad sense heritability was estimated by comparing the segregating and homogenous population as follow:

$$H=\frac{V_g}{V_p}x100$$

Where;

H= Broad sense heritability

V_g= Genotypic variance

V_p= Phenotypic variance = Variance of F_2.

Genotypic variance was estimated by subtracting the environmental variance (V_e) from the phenotypic variance (Variance of F_2).

Environmental variance was estimated as follows:

$$Environmental\ variance(V_e)=\frac{V_{p1}+V_{p2}+V_{F1}(Cross)+V_{F1}(Rec.)}{4}$$

Where;

V_{p1}= Variance of first parent

V_{p2}= Variance of second parent

V_{F1}(Cross)= Variance of F_1 (Cross)

V_{F1}(Rec.) = Variance of F_1 (Reciprocal)

4. RESULT AND DISCUSSION

4.1 Low ODAP X low ODAP lines

Four low ODAP lines L720060, L900436, LS82046 and LS90235 were inter-crossed (crosses and reciprocals) to evaluate the genetic basis of low seed ODAP concentration in grass pea. In general, progenies of the low ODAP lines did not segregate for high ODAP concentration suggesting common genes among the low lines in grass pea. However, F_2 progenies had a wider variability compared to parental and F_1 population which might suggest the presence of different modifier genes among the lines. Cytoplasmic influence was detected in 2 of the low lines. The progenies of the line L720060 segregated for low ODAP concentration when used as the female parent as compared to using it as the male parent (Figure 4.1a). In contrast, the progenies of the line LS82046 segregated for high ODAP when utilized as the female parent (Figure 4.1b). The line LS90235 showed dominant nuclear genetic effect that masked the cytoplasmic influence of other lines (Figure 4.1c). Quader *et al.* (1987) reported ODAP to be influenced by a cytoplasmic effect.

4.1.1 Line L720060[2] x line L900436

Both parents had low ODAP concentration. Parental line L720060

[2]First line in a cross is the female parent.

had a mean ODAP concentration of 0.551±0.038 and line L900436 0.338±0.025 mg/g ground seed at zero % moisture (Table 4.1.1a). Mean ODAP concentration of progenies, both of the cross and the reciprocal, were intermediate. F_1 progenies of the cross L720060 x L900436 produced a mean ODAP concentration of 0.353±0.068 while the reciprocal cross yielded a mean ODAP concentration of 0.464±0.062 mg/g of seed. The mean ODAP concentration of F_2 progenies were significantly different between the cross and the reciprocal. F_2 progenies of the cross between L720060 x L900436 produced a mean ODAP concentration of 0.497±0.026 mg/g of seed as compared to 1.666±0.059 mg/g which is significantly higher than its reciprocal (Table 4.1.1a). The broad sense heritability was estimated to be 93%.

The frequency distribution of ODAP concentration of the parents, F_1 and F_2 progenies is presented in Table 4.1.1b. The two parents were distributed similarly in the ODAP concentration. Individual F_1 plant distribution for ODAP concentration was in the intermediate range. Wide variation in ODAP concentration was noted in the F_2 progenies, particularly when L900436 was used as the female parent. Variation in both the F_2 progenies was continuous and the distribution of the cross L720060 x L900436 appeared to be normal. It was not possible to distinguish discrete phenotypic classes of low or high ODAP. The genetic characters contributing to the

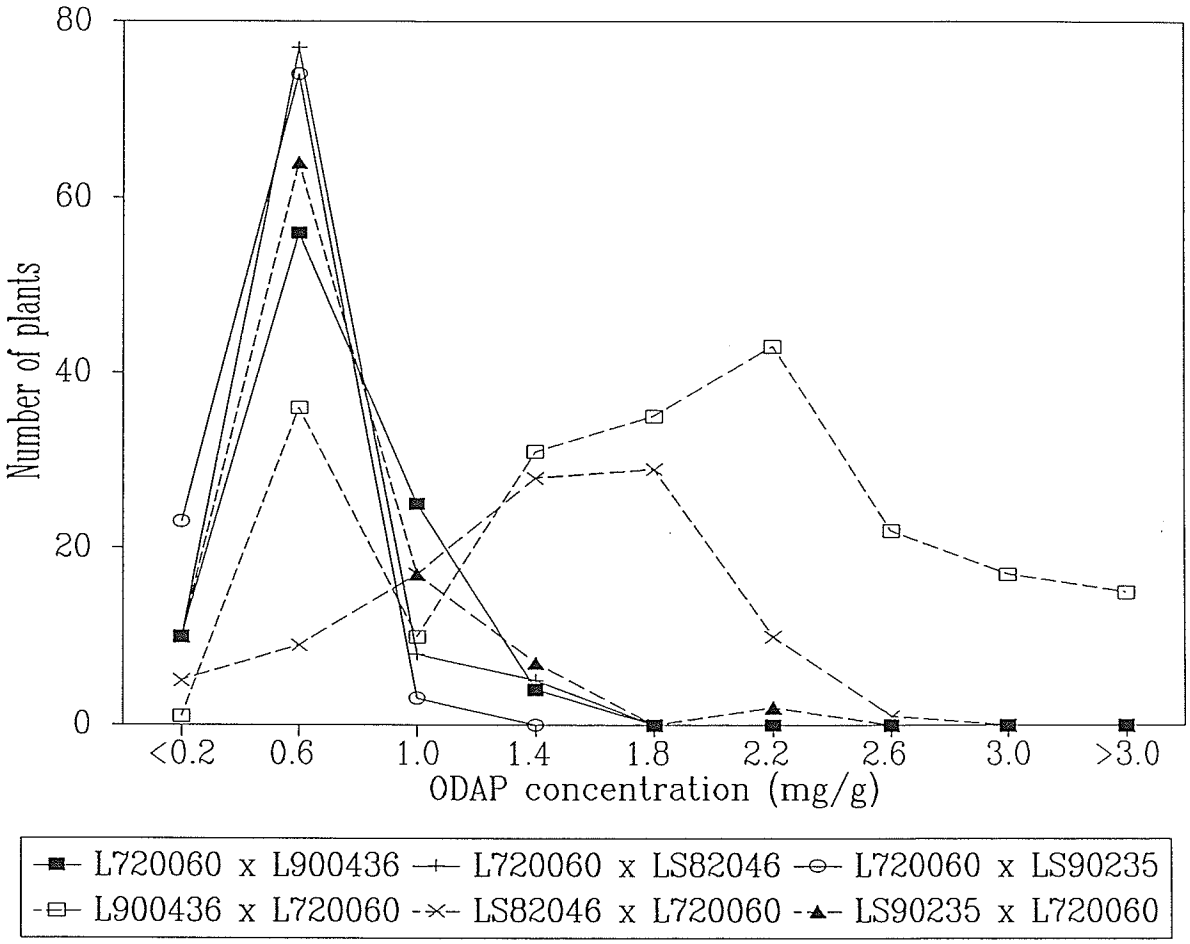


Figure 4.1a. Distribution of F_2 progenies on seed ODAP concentration when line L720060 was used as female or male parent.

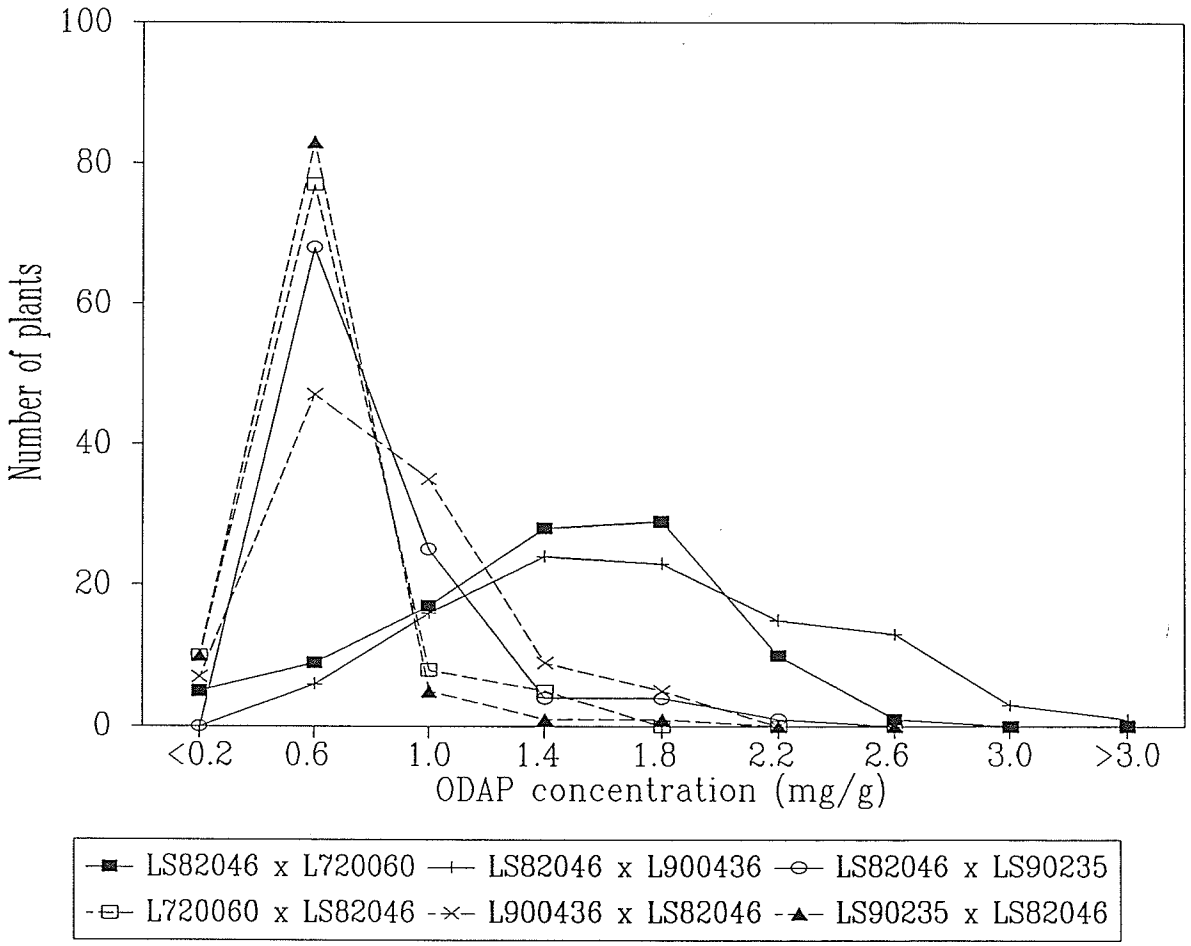


Figure 4.1b. Distribution of F_2 progenies on seed ODAP concentration when line LS82046 was used as male or female parent.

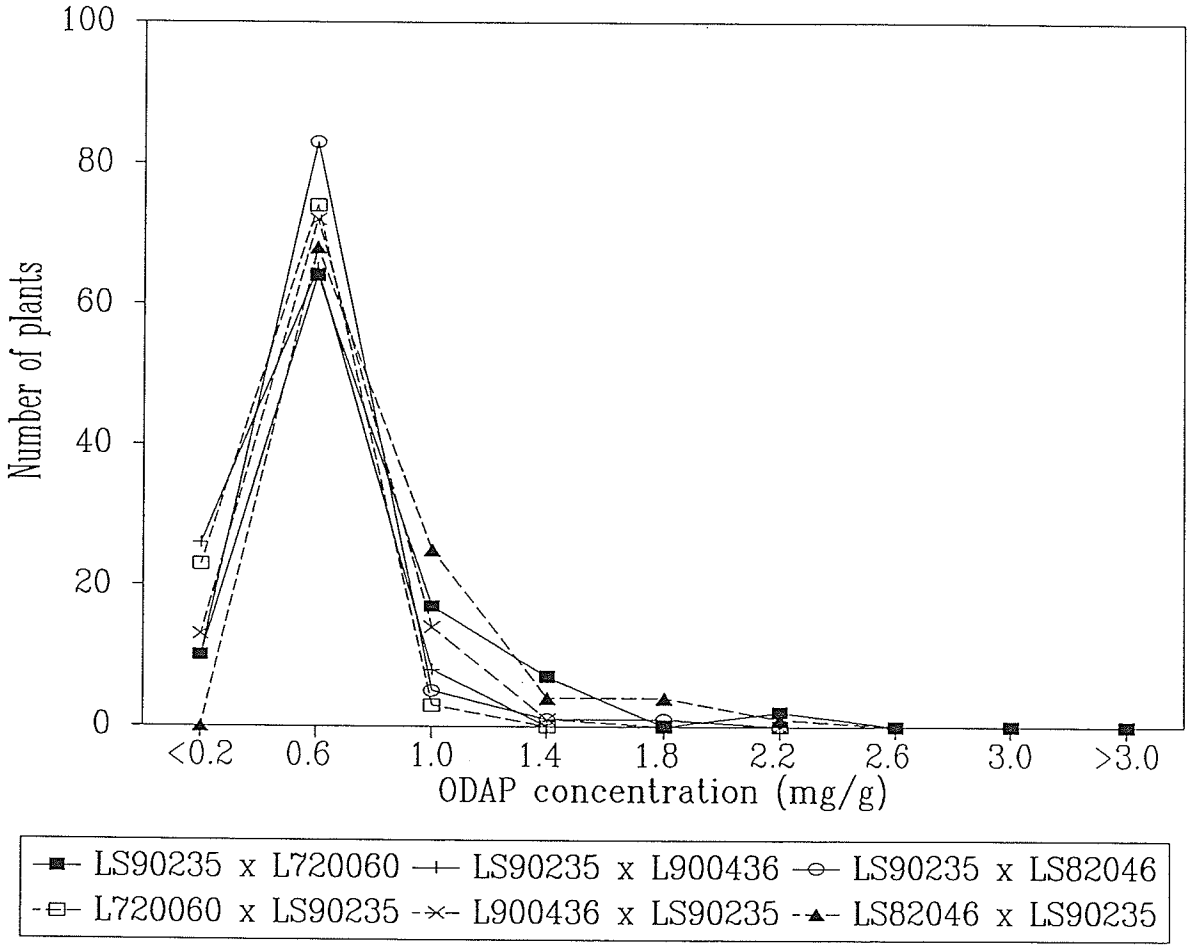


Figure 4.1c. Distribution of F_2 progenies on seed ODAP concentration when line LS90235 was used as male or female parent.

Table 4.1.1a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. L720060	9	0.395-0.713	0.551	0.038	0.114	20
2. L900436	10	0.192-0.426	0.338	0.025	0.080	23
3. F1(1X2)	10	0.115-0.722	0.353	0.068	0.216	61
4. F1(2X1)	10	0.212-0.866	0.464	0.062	0.197	42
5. F2(1X2)	95	0.086-1.310	0.497	0.026	0.261	53
6. F2(2X1)	210	0.163-3.997	1.666	0.059	0.864	52

Heritability (H)% = 93

T test:
F_1 means: ns
F_2 means: **

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.1.1b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. L720060		5	4						
2. L900436	1	9							
3. F1(1X2)	3	4	3						
4. F1(2X1)		8	2						
5. F2(1X2)	10	56	25	4					
6. F2(2X1)	1	36	10	31	35	43	22	17	15

Values are the number of plants in the range.

concentration of ODAP in the seed between these two low lines might possibly be similar.

The mean ODAP concentration of the F_1 progenies both for the cross and the reciprocal was in the parental range, indicated an absence of heterosis for seed ODAP concentration. When line L720060 was used as the female parent similar concentration of ODAP as that of the parents and F_1 progenies was detected in the F_2 progenies. However, the results differed when line L900436 was used as the maternal parent. The F_2 progenies segregated for high ODAP concentration in this cross. An involvement of a cytoplasmic factor could be a possibility when line L900436 was used as the female parent. However, there was no detectable cytoplasmic influence in the F_1 progenies. ODAP is reported to be highly influenced by environmental factors (Kaul *et al.*, 1986; Campbell, 1989; Bell, 1993), which could be another possible reason for higher variability found between the cross and the reciprocal. Campbell (1992) reported that 15 to 20% out crossing could occur in grass pea under field conditions. Seed for the F_2 progenies were grown out as F_1 plants in the field and out crossing could be another possible factor to increase the observed ODAP variability. Two peaks in the distribution of ODAP in the F_2 progenies indicated the presence of allelism between these two low lines indicating simple Mendelian inheritance as reported by Quader (1985) with dominant action

of high ODAP. However, the continuous and normal distribution found in the reciprocal cross does not corroborate these results. A broad sense heritability estimation of 93% in this cross, indicated that there was minimal environmental effect on ODAP concentration.

4.1.2 Line L720060 x line LS82046

Both parents of this cross were low in ODAP concentration. Line L720060 and line LS82046 produced a very similar level of mean ODAP concentration of 0.551±0.038 and 0.526±0.025 mg/g of seed respectively. A significantly higher mean ODAP concentration of 1.090±0.131 mg/g of seed was produced in F_1 progenies when line LS82046 was used as the female parent as compared to 0.572±0.109 for its reciprocal. As in the F_1 progenies, a significantly higher mean ODAP concentration of 1.199±0.052 mg/g of seed was produced in the F_2 progenies when line LS82046 was used as the female parent as compared to mean ODAP concentration of 0.399±0.022 mg/g seed of reciprocal cross (Table 4.1.2a). Thus, it would appear that line LS82046 contributed to a higher ODAP concentration when this line was used as the female parent. A broad sense heritability of 58% was estimated.

The frequency distribution on the ODAP concentration by individual plants of parents, F_1 progenies and F_2 progenies is

Table 4.1.2a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	C V %
1. L720060	9	0.395-0.713	0.551	0.038	0.114	20
2. LS82046	8	0.443-0.674	0.526	0.025	0.073	14
3. F1(1X2)	7	0.202-0.876	0.572	0.109	0.289	50
4. F1(2X1)	10	0.529-1.710	1.090	0.131	0.414	38
5. F2(1X2)	100	0.115-1.165	0.399	0.022	0.220	55
6. F2(2X1)	99	0.105-2.272	1.199	0.052	0.519	44

Heritability (H) % = 58

T test:
F_1 means: *
F_2 means: **

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.1.2b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. L720060		5	4						
2. LS82046		7	1						
3. F1(1X2)		3	4						
4. F1(2X1)		2	2	3	3				
5. F2(1X2)	10	77	8	5					
6. F2(2X1)	5	9	17	28	29	10	1		

Values are the number of plants in the range.

presented in Table 4.1.2b. The two parents showed very small variability and were distributed across a similar range (0.6 to 1.0 mg/g). The F_1 progenies were also distributed over the same range with a slightly higher variability when the line LS82046 was used as the female parent. A wider variability was observed in ODAP concentration of the F_2 progenies, particularly when the line LS82046 was used as the female parent. Variation in ODAP concentration of F_2 progenies was continuous and normally distributed. Classification of this data into discrete phenotypic classes of low and high ODAP was not possible indicating quantitative inheritance.

The low ODAP parents produced F_1 and F_2 progenies with low ODAP concentration when line L720060 was used as the female parent. A significantly higher mean ODAP seed concentration was found in the F_1 and F_2 progenies when LS82046 used as the female parent which might indicate the involvement of a cytoplasmic factor. Campbell (1992) reported that the low ODAP character of LS82046 could be associated with another character preventing normal expression of the anthers. Out crossing in the field with high ODAP content parents may have contaminated the genetic variability expressed by this line. He also reported on 15 to 16% out crossing both in Canada and Bangladesh under field conditions. A cytoplasmic character could also be associated with increased ODAP production when this line was used as the female parent.

4.1.3 Line L720060 x line LS90235

The line L720060 and LS90235 both produce a low ODAP concentration with mean ODAP of 0.551±0.038 and 0.667±0.050 mg/g of seed respectively (Table 4.1.3a). The mean ODAP concentration of F_1 progenies in the cross of L720060 x LS90235 and the reciprocal was 0.414±0.057 and 0.497±0.052 mg/g of seed respectively. A F_2 mean ODAP concentration of 0.305± 0.014 mg/g of seed was found when L720060 was used as the female parent and 0.503±0.033 mg/g seed was found when the line L720060 was used as the male parent (Table 4.1.3a). Both parental lines LS90235 and L720060 produced a lower mean ODAP concentration in the F_1 and F_2 progenies as compared to the parental mean value. The broad sense heritability was estimated to be 70%.

The frequency distribution of individual plants for ODAP concentration of parents, F_1 progenies and F_2 progenies has been presented in Table-4.1.3b. Both parents had a similar distribution range including the F_1 progenies as well. The F_2 progenies were more variable than the parental and F_1 progenies. However, variation of ODAP concentration in F_2 progenies was found to be continuous and normally distributed. Phenotypic classification of the data into discrete classes of low and high ODAP concentration was not possible. Genetic characters contributing to seed ODAP concentration might possibly be similar in these two low lines.

Table 4.1.3a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. L720060	9	0.395-0.713	0.551	0.038	0.114	20
2. LS90235	11	0.472-1.021	0.677	0.050	0.166	24
3. F1(1X2)	5	0.192-0.578	0.414	0.057	0.129	31
4. F1(2X1)	10	0.279-0.876	0.497	0.052	0.166	33
5. F2(1X2)	100	0.057-0.874	0.305	0.014	0.137	45
6. F2(2X1)	100	0.115-2.118	0.503	0.033	0.338	67

Heritability (H)% = 70

T test:
F_1 means: ns
F_2 means: **

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.1.3b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration							
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. L720060		5	4						
2. LS90235		5	5	1					
3. F1(1X2)	1	4							
4. F1(2X1)		9	1						
5. F2(1X2)	23	74	3						
6. F2(2X1)	10	64	17	7		2			

Values are number of plants in the range.

The low ODAP parents, F_1 and F_2 progenies, all in the low ODAP range, indicated the absence of genetic polymorphism. Both parents were originated from France and segregation for high ODAP was not found. Possibly these two lines share common genetic characters for seed ODAP concentration. Though mean F_2 ODAP concentration between the cross and the reciprocal indicated significant differences this possibly may be attributed to environmental variations.

4.1.4 Line L900436 x line LS82046

Both low ODAP parents L900436 and LS82046, with a mean ODAP concentration of 0.338±0.025 and 0.526±0.025 mg/g respectively, were used in this cross. The F_1 progenies of the cross L900436 x LS82046 produced a mean ODAP concentration of 0.522±0.053 mg/g with a range of 0.375 to 0.818 mg/g seed whereas the reciprocal cross produced a significantly higher ODAP concentration of 0.860±0.073 mg/g of seed ranging from 0.645 to 1.250 mg/g of seed. A significantly higher mean ODAP concentration was determined in the F_2 progenies when the line LS82046 was used as the female parent as compared to the reciprocal. The mean ODAP concentration of 0.625±0.034 was found when the line L900436 was utilized as the female parent, with a range of 0.038 to 1.502, whereas the reciprocal cross produced a significantly higher mean ODAP concentration of 1.540±0.064 mg/g of seed with a range of 0.308 to 3.226 mg/g (Table 4.1.4a). A high broad sense heritability of 92% was

Table 4.1.4a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. L900436	10	0.192-0.426	0.338	0.025	0.080	23
2. LS82046	8	0.443-0.674	0.526	0.025	0.073	14
3. F1(1X2)	9	0.375-0.818	0.522	0.053	0.159	30
4. F1(2X1)	8	0.645-1.250	0.860	0.073	0.207	24
5. F2(1X2)	103	0.038-1.502	0.625	0.034	0.351	56
6. F2(2X1)	101	0.308-3.226	1.540	0.064	0.644	42

Heritability (H) % = 92

T test:
F_1 means: **
F_2 means: **

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.1.4b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. L900436	1	9							
2. LS82046		7	1						
3. F1(1X2)		7	2						
4. F1(2X1)			5	3					
5. F2(1X2)	7	47	35	9	5				
6. F2(2X1)		6	16	24	23	15	13	3	1

Values are the number of plants in the range.

estimated for this cross indicating this trait to be highly heritable.

A frequency distribution of individual plants on ODAP concentration of the parents, F_1 progenies and F_2 progenies is presented in Table 4.1.4b. The parental lines resembled each other in distribution of ODAP concentration. The distribution of both the parents and the F_1 progenies was quite narrow, whereas the distribution of the F_2 population was quite variable. Variation in both the F_2 populations were continuous fitting very close to a normal distribution. However, both in the cross and reciprocal the distribution curve was slightly skewed to the right.

The low ODAP concentration of F_1 progenies indicated the absence of genetic polymorphism for seed ODAP between the two low ODAP lines. Possibly, these two low lines might contain a similar genetic make up for low seed ODAP concentration. However, a significantly higher seed ODAP concentration both in the F_1 and F_2 progenies when LS82046 was used as the female parent could possibly be due to a cytoplasmic effect of the line LS82046. Higher seed ODAP concentration was similarly found in other cross combinations when the line LS82046 was used as the female parent. Continuous variation together with normally distributed F_2 population indicated this trait to be inherited quantitatively.

4.1.5 Line L900436 x line LS90235

Both the parental lines contain low but different levels of ODAP concentration. Line L900436 contained 0.338±0.025 and LS90235 contained a higher level at 0.677±0.050 mg/g of seed. The F_1 progenies of the cross L900436 x LS90235 produced a mean ODAP concentration of 0.476±0.056 with a range of 0.231 to 0.683 mg/g. However, the reciprocal cross produced significantly higher ODAP at 0.914±0.088 mg/g of seed with a range of 0.693 to 1.271 mg/g of seed. The mean ODAP concentration of the F_2 progenies of the cross L900436 x LS90235 was 0.374±0.019 (range: 0.144 to 1.146) mg/g of seed as compared to the reciprocal cross of 0.324±0.018 (range 0.048 to 0.973) mg/g of seed (Table 4.1.5a). A relatively low broad sense heritability of 23% was estimated for this cross.

The frequency distribution of ODAP concentration of the parents, F_1 and F_2 progenies is presented in Table 4.1.5b. Both parents were distributed in the range of 0.2 to 1.0 mg /g of seed. The F_1 progenies were in the parental range. The distribution of the F_2 population also was in the parental range, lacking segregation for high ODAP. However, variation was continuous and normally distributed. Classification of the data on low and high phenotypic classes was not possible suggesting similar genetic characters contributing to the seed concentration of the neurotoxin ODAP.

Table 4.1.5a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. L900436	10	0.192-0.426	0.338	0.025	0.080	23
2. LS90235	11	0.472-1.021	0.677	0.050	0.166	24
3. F1(1X2)	7	0.231-0.683	0.476	0.056	0.148	31
4. F1(2X1)	7	0.693-1.271	0.914	0.088	0.233	25
5. F2(1X2)	100	0.144-1.146	0.374	0.019	0.192	51
6. F2(2X1)	99	0.048-0.973	0.324	0.018	0.181	56

Heritability (H) %= 23

T test:
F_1 means: **
F_2 means: ns

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.1.5b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. L900436	1	9							
2. LS90235		5	5	1					
3. F1(1X2)		5	2						
4. F1(2X1)			5	2					
5. F2(1X2)	13	72	14	1					
6. F2(2X1)	26	65	8						

Values are the number of plants in the range.

The low ODAP parental lines and the F_1 progenies with low seed ODAP concentration indicated the absence of heterosis for seed ODAP concentration. Though, the F_1 mean for ODAP concentration were significantly different, the small number of observations of F_1 progenies and the high environmental influence on ODAP might possibly be the reason for this difference. The ODAP concentration of the F_2 progenies showed a continuous variation and very close to normal distribution, characteristic of quantitative inheritance. A broad sense heritability of 23% also indicated that there was high environmental influence on the expression of this trait.

4.1.6 Line LS82046 x line LS90235

The low ODAP lines LS82046 (mean ODAP 0.526±0.025) and LS90235 (mean ODAP: 0.677±0.050) were used in this cross to evaluate the genetic situation (Table 4.1.6a). The mean ODAP concentration of the F_1 progenies was found to be higher than either parental means. The F_1 progenies of the cross LS82046 x LS90235 produced a mean ODAP concentration of 0.821±0.111 and the reciprocal produced a similar mean ODAP of 0.825±0.117 mg/g. A significantly higher mean ODAP concentration of 0.595±0.032 mg/g was produced when the line LS82046 was used as the female parent as compared to 0.385±0.019 mg/g for its reciprocal in the F_2 progenies (Table 4.1.6a). A broad sense heritability of ODAP concentration in this cross was estimated at 17%.

The frequency distribution of individual plants for ODAP concentration of the parents, F_1 and F_2 progenies is presented in Table 4.1.6b. The two parents were very similar for ODAP concentration. Higher variability was exhibited by the F_1 progenies when compared to the parental lines, particularly in the higher ODAP range. The F_2 population was more variable indicating both genotypic and environmental variation. Variation was continuous in both of the F_2 populations. Frequency distribution for the cross LS90235 x LS82046 was very close to normal, however, the reciprocal was skewed to the right with no distribution in the lower range which could be mainly due to the involvement of a cytoplasmic factor when line LS82046 was used as the female parent. Phenotypic classification of the segregating F_2 population to low and high ODAP could not be made hence the genetic characters controlling the seed ODAP concentration were expected to be similar in these two low ODAP lines.

The two low ODAP parental lines had similar ODAP concentration. A higher seed ODAP concentration of the F_1 progenies both in the cross and the reciprocal could possibly be due to the expression of heterosis or simply due to environmental influence because of the small number of samples analyzed in the F_1 progenies. In the F_2 progenies, significantly higher mean seed ODAP was produced when the line LS82046 was used as the female parent as the observation found

Table 4.1.6a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. LS82046	8	0.443-0.674	0.526	0.025	0.073	14
2. LS90235	11	0.472-1.021	0.677	0.050	0.166	24
3. F1(1X2)	8	0.433-1.367	0.821	0.111	0.313	38
4. F1(2X1)	10	0.481-1.502	0.825	0.117	0.371	45
5. F2(1X2)	102	0.231-1.820	0.595	0.032	0.322	54
6. F2(2X1)	100	0.096-1.425	0.385	0.019	0.198	51

Heritability (H) % = 17

T test:
F_1 means: ns
F_2 means: **

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.1.6b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. LS82046		7	1						
2. LS90235		5	5	1					
3. F1(1X2)		2	3	3					
4. F1(2X1)		5	2	2	1				
5. F2(1X2)		68	25	4	4	1			
6. F2(2X1)	10	83	5	1	1				

Values are the number of plants in the range.

in other cross combinations. These two low ODAP lines might contain similar genetic make up for seed ODAP concentration with the exception of the cytoplasmic influence of the line LS82046 toward higher ODAP concentration. A broad sense heritability of 17% in this cross indicated that this trait would be highly influenced by the environmental factors.

4.2. Low ODAP X High ODAP lines

The four low ODAP lines L720060, L900436, LS82046 and LS90235 were crossed to the high ODAP line L880283 to observe resulting segregation patterns. All the low ODAP lines were well separated from the high ODAP line in ODAP concentration. The F_1 progenies of the low ODAP x high ODAP lines were found to be in the intermediate range. The F_2 progenies segregated covering the entire parental range (Figure 4.2.). A significantly higher seed ODAP concentration was detected when the high ODAP line L880283 was used as the female parent both in the F_1 and F_2 progenies of the cross L880283 x L720060 and in the F_2 progenies of the cross L880283 x L900436 and L880283 x LS90235. Similar to this observation, *Quader et al.* (1987) reported cytoplasmic influence on ODAP concentration.

4.2.1 Line L720060 x line L880283

Line L720060 contained low ODAP concentration of 0.551±0.038 while the line L880283 contained a high ODAP concentration of 2.540±0.082 mg/g (Table 4.2.1a). The F_1 progenies of both the

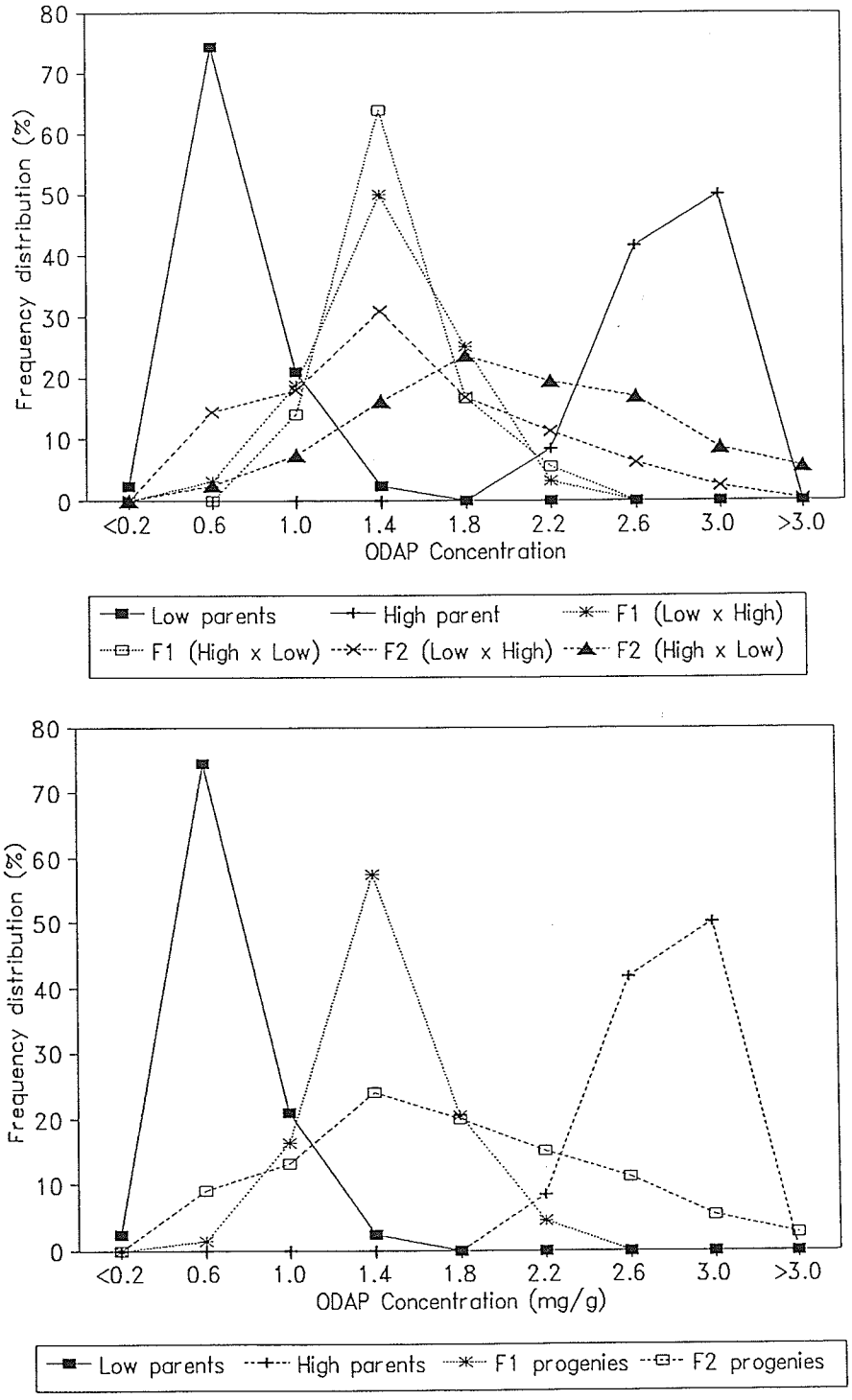


Figure 4.2 Distribution of seed ODAP concentration in parents, F_1 and F_2 progenies in low ODAP x high ODAP crosses.

Table 4.2.1a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. L720060	9	0.395-0.713	0.551	0.038	0.114	20
2. L880283	12	1.983-2.995	2.540	0.082	0.286	11
3. F1(1X2)	5	0.433-1.350	0.876	0.130	0.310	35
4. F1(2X1)	4	1.300-2.195	1.750	0.150	0.316	18
5. F2(1X2)	102	0.385-2.407	1.183	0.040	0.405	34
6. F2(2X1)	100	1.136-3.621	2.385	0.049	0.494	21

Heritability (H)% = 65

T test:
F_1 means: *
F_2 means: **

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.2.1b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. L720060		5	4						
2. L880283						1	5	6	
3. F1(1X2)		1	2	2					
4. F1(2X1)				1	2	1			
5. F2(1X2)		9	22	48	15	7	1		
6. F2(2X1)				2	9	21	34	23	11

Values are the number of plants in the range.

cross and the reciprocal were in the intermediate range in mean ODAP concentration. However, the F_1 progenies of L880283 x L720060 produced a significantly higher mean ODAP level of 1.750±0.150 mg/g of seed than the reciprocal cross with a mean ODAP concentration of 0.876±0.130 mg/g. Similarly, the F_2 progenies also exhibited significantly different mean ODAP production when the high parent L880283 was used as male or female parent. The F_2 progenies of the cross L720060 x L88283 produced a mean ODAP concentration of 1.183±0.040 as compared to 2.385± 0.049 mg/g when the low parent L720060 was used as the male parent (Table 4.2.1a). A possible involvement of a cytoplasmic factor could influence the ODAP content in this cross producing a higher ODAP concentration when the high ODAP parent was used as the female parent. The broad sense heritability value in this cross was estimated to be 65%.

The distribution of ODAP concentration of individual plants of parents, F_1 progenies and F_2 progenies is presented in Table 4.2.1b. The parental distributions in this cross were well separated in ODAP concentration. The F_1 progenies of the cross and the reciprocal were found to be in the intermediate range. The F_1 progenies of L880283 x L720060 were in the upper range in mean ODAP concentration as compared to the reciprocal cross which were in the lower range. The distribution of ODAP concentration of the F_2 progenies was highly variable overlapping both the low and high parental ranges.

Distribution of ODAP concentration of the F_2 progenies was in the upper range when the high ODAP parental line L880283 was used as the female and was in the lower range when the low ODAP parent L720060 was used as the female. In both cases, the variation was continuous and normally distributed. Classification of the F_2 data for discrete classes of low and high ODAP concentration was not possible. The continuous variation of the segregating F_2 progenies together with the normal distribution found indicated that ODAP concentration was inherited quantitatively.

A significantly higher seed ODAP concentration of F_1 and F_2 progenies when high ODAP line L880283 used as the female parent, revealed a maternal effect for seed ODAP. Similar to our observation, Quader (1987) also reported that ODAP content although determined by the parental and filial genetic constitution, is also influenced by a cytoplasmic effect. Intermediate distribution of ODAP concentration in the F_1 indicated the absence of dominance either by low or high ODAP concentration, a typical characteristic of a quantitative trait. This observation was not consistent with the findings of Nerkar (1972) and Quader (1985) who reported three distinct peaks in the F_2 distribution and postulated a monogenic inheritance. However, Dahiya (1986) reported ODAP to be quantitatively inherited. The number of genes involved in the production of seed ODAP concentration could be many.

4.2.2 Line L900436 x line L880283

The low ODAP line L900436 (0.338±0.025) was crossed with the high ODAP line L880283 (2.540±0.082) (Table 4.2.2a). The F_1 progenies were in the intermediate range indicating no dominance relationship. The mean ODAP concentration of the F_1 of the cross L900436 x L880283 was 1.229±0.067 and that of the reciprocal was 1.132±0.049 mg/g of seed. The mean ODAP concentration of the F_2 progenies were also found to be in the intermediate range. F_2 progenies of the cross L900436 x L880283 produced a mean ODAP concentration of 1.137±0.041 mg/g while the reciprocal cross produced a mean ODAP concentration of 1.513±0.067 mg/g (Table-4.2.2a). The broad sense heritability was estimated to be 88%.

The frequency distribution of individual plants for ODAP concentration of the parents, F_1 progenies and the F_2 progenies is presented in Table 4.2.2b. The distribution of the parents for ODAP production were well separated. The F_1 plants were distributed in an intermediate range. Variability of both parental lines and the F_1 progenies were low as seen in the frequency distribution (Table-4.2.2b). Both F_2 progenies exhibited a large variability overlapping both the low and high parental ranges. The distribution of the F_2 progenies, both of the cross and the reciprocal was continuous and normally distributed with slight skewing to the right.

Table 4.2.2a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. L900436	10	0.192-0.426	0.338	0.025	0.080	23
2. L880283	12	1.983-2.995	2.540	0.082	0.286	11
3. F1(1X2)	13	0.828-1.579	1.229	0.067	0.243	19
4. F1(2X1)	12	0.886-1.464	1.132	0.049	0.171	15
5. F2(1X2)	164	0.250-2.687	1.137	0.041	0.530	47
6. F2(2X1)	101	0.327-3.438	1.513	0.067	0.672	45

Heritability (H) % = 88

T test:
F_1 means: ns
F_2 means: **

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.2.2b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. L900436	1	9							
2. L880283						1	5	6	
3. F1(1X2)			2	7	4				
4. F1(2X1)			2	8	2				
5. F2(1X2)		31	34	54	22	18	4	1	
6. F2(2X1)		6	13	33	21	12	9	2	5

Values are the number of plants in the range.

Separation of the resultant data into low and high phenotypic classes was not possible. This continuous variation coupled with the normal distribution of the segregating F_2 progenies indicated that ODAP concentration was quantitatively inherited.

The intermediate seed ODAP concentration of the F_1 progenies indicated the lack of dominance either by high or low seed ODAP concentration. A significantly higher mean seed ODAP concentration in the F_2 progenies when the high ODAP line L880283 was used as the female parent could be associated with a cytoplasmic influence. However, cytoplasmic influence was not evident in the mean ODAP concentration of the F_1 progenies. The other possible factor could be environmental influences which could be quite high as reported by Kuo (1990) and Campbell (1990). The F_2 progenies could not be classified into discrete phenotypic classes of low and high seed ODAP concentration though parents were well separated. High variability of F_2 progenies as compared to parents and F_1 progenies represented both genotypic and environmental variation whereas variation in parents and F_1 progenies was attributed to the environmental influence. A high heritability of 88% indicated that environmental effects were small.

4.2.3 Line LS82046 x line L880283

The low ODAP line LS82046 (mean ODAP concentration of

0.526±0.025) was crossed with the high ODAP line L880283 (mean ODAP of 2.540±0.082). Mean ODAP concentration of the F_1 progenies was in the intermediate range indicating lack of dominant inheritance for low or high ODAP. The F_1 progenies of LS82046 x L880283 produced a mean ODAP concentration of 1.262±0.101 mg/g and the reciprocal produced 1.233±0.056 mg/g of seed. No cytoplasmic influence was exhibited in either F_1 or F_2 progenies in this cross. The mean ODAP concentration of the F_2 progenies was in the intermediate range of the parental lines. The mean ODAP concentration of the F_2 progenies when the low ODAP line LS82046 was used as the female was 1.607±0.073 and 1.698±0.0667 mg/g when the high line L880283 was used as the female (Table 4.2.3a). The broad sense heritability was estimated to be 90%.

A frequency distribution of individual plants for ODAP concentration of parental lines, F_1 progenies and F_2 progenies has been presented in Table 4.2.3b. Parental lines ODAP distribution was widely separated. All the F_1 plants were found in the intermediate range. Variability was found to be quite low in both parental lines and F_1 progenies. The F_2 population was quite variable exhibiting both genotypic and the environmental variation covering the entire parental range. Distribution of the F_2 progenies was continuous and normally distributed. This continuous variation with normally

Table 4.2.3a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. LS82046	8	0.443-0.674	0.526	0.025	0.073	14
2. L880283	12	1.983-2.995	2.540	0.082	0.286	11
3. F1(1X2)	8	0.731-1.570	1.262	0.101	0.287	22
4. F1(2X1)	10	0.963-1.569	1.233	0.056	0.178	14
5. F2(1X2)	97	0.385-2.985	1.607	0.073	0.728	45
6. F2(2X1)	101	0.462-3.600	1.698	0.067	0.680	40

Heritability (H)% = 90

T test:
F_1 means: ns
F_2 means: ns

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.2.3b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. LS82046		7	1						
2. L880283						1	5	6	
3. F1(1X2)			2	3	3				
4. F1(2X1)			2	6	2				
5. F2(1X2)		11	14	13	20	15	15	9	
6. F2(2X1)		4	15	15	29	11	17	7	3

Values are the number of plants in the range.

distributed segregating F_2 population could most probably be indicative of quantitative inheritance.

The low and high ODAP parents were well separated in seed ODAP concentration. The F_1 progenies of both the cross and the reciprocal indicated the lack of dominance either by low or high parents giving intermediate values with additive genetic effects. Non significant differences between the cross and the reciprocal, both in the F_1 and F_2 progenies, indicated the absence of any cytoplasmic influence or that which could be associated with the cytoplasmic influence of low ODAP line LS82046 to give high ODAP progenies. Distribution of individual F_2 progenies showed high variability representing both the high and the low parental range. The higher variability of the F_2 progenies could be an indication of genetic variability and environmental effect, whereas, the parents and the F_1 progenies indicated only the environmental variations. A fairly high heritability value of 90% indicated this trait to be highly heritable.

4.2.4 Line LS90235 x line L880283

The low ODAP line LS90235 (0.677±0.050) was crossed with the high ODAP line L880283 (2.540±0.082) (Table 4.2.4a). The mean ODAP concentration of the F_1 progenies were in the intermediate range which indicated a lack of dominance for either low or high ODAP concentration. The F_1 progenies of the

cross LS90235 x L880283 produced a mean ODAP concentration of 1.409±0.141 and that of the reciprocal was 1.257±0.085 mg/g of seed. The ODAP concentration in F_1 of the cross and the reciprocal indicated that a cytoplasmic effect was not evident. The mean ODAP concentration of the F_2 progenies were in the intermediate range of the parental lines. The F_2 progenies of the cross LS90235 x L880283 produced a mean ODAP concentration of 1.334±0.059 mg/g, with the progenies of reciprocal cross producing a significantly higher mean ODAP concentration of 1.786±0.045 mg/g of seed (Table 4.2.4a). Broad sense heritability was estimated to be 71%.

The frequency distribution of individual plants by ODAP concentration of the parental lines, F_1 progenies and F_2 progenies is presented in Table 4.2.4b. The distribution of parental lines did not overlap in ODAP production. All the F_1 plants were found to be in the intermediate range. Variability was quite low both in the parental lines and in the F_1 progenies. Progenies of both the F_2 cross and reciprocal populations were quite variable and in the range of both the low and high parental lines. There was no discrete classes in the distribution of ODAP concentration and thus the F_2 progenies exhibited continuous variation. As distribution was very close to normal this indicates that several genes are involved in ODAP concentration. Thus the continuous variation

Table 4.2.4a. Simple statistics on ODAP (mg/g of seed) of parents, F_1 and F_2 progenies in grass pea.

Cross	N	Range	Mean	SE	SD	CV%
1. LS90235	11	0.472-1.021	0.677	0.050	0.166	24
2. L880283	12	1.983-2.995	2.540	0.082	0.286	11
3. F1(1X2)	6	1.059-2.000	1.409	0.141	0.345	24
4. F1(2X1)	11	0.992-1.964	1.257	0.085	0.284	22
5. F2(1X2)	101	0.289-2.697	1.334	0.059	0.590	44
6. F2(2X1)	100	0.789-3.332	1.786	0.045	0.457	26

Heritability (H) % = 71

T test:
F_1 means: ns
F_2 means: **

N=Number of Plants, SE=Standard Error, SD=Standard Deviation and CV=Coefficient of Variation. ns=Non Significant, * and ** =Significantly different at 0.05 and 0.01 level of probability respectively.

Table 4.2.4b. Frequency distribution of ODAP concentration (mg/g of seed) on parents, F_1 and F_2 generation.

	ODAP Concentration								
Cross	<0.2	0.2-0.6	0.6-1.0	1.0-1.4	1.4-1.8	1.8-2.2	2.2-2.6	2.6-3.0	>3.0
1. LS90235		5	5	1					
2. L880283						1	5	6	
3. F1(1X2)				4	1	1			
4. F1(2X1)			1	8	1	1			
5. F2(1X2)		16	13	28	21	13	8	1	1
6. F2(2X1)			2	15	36	34	8	2	3

Values are the number of plants in the range.

and the normally distributed F_2 population indicated that ODAP seed concentration was most probably inherited quantitatively.

The two low and high ODAP parents produced F_1 progenies with intermediate seed ODAP concentration with additive genetic effect. Involvement of a cytoplasmic factor was not evident on the mean ODAP concentration of F_1 progenies with similar mean ODAP concentration of the cross and the reciprocal. However, mean ODAP concentration of the F_2 progenies, was significantly higher when the high ODAP parent, L880283 was used as the female parent which could possibly due to a cytoplasmic influence. The small number of samples analyzed in the F_1 progenies could possibly be the reason of the inability to detect cytoplasmic influence in F_1 progenies. Distribution of the F_2 population represented both the high and low parental range (wider variation) indicating higher genotypic differences than that of the parental and F_1 populations.

4.3 Year effect

Line LS90043 was planted as a check variety both experimental years to evaluate effect of years on the concentration of ODAP. Twenty-five randomly selected individual plants were analyzed each year for ODAP concentration. The mean ODAP concentration between the years was non significant which indicated that years were not significantly different in mean seed ODAP concentration. Though, environment has been reported

to influence ODAP concentration, this non significant difference could be due to the occurrence of cool and wet weather conditions during both experimental years.

5. GENERAL DISCUSSION

Nerkar (1972) reported that ODAP content might exhibit a simple Mendelian inheritance from variation induced in *Lathyrus* from the study of segregating M_2 generations. However, variation was continuous though 3 peaks were present and the choice of the phenotypic class interval was somewhat arbitrary, which also could make a difference in such studies. In Quader's report (1985), variations of the F_2 progenies was also continuous, though he postulated a simple monogenic inheritance on the basis of peaks. However, results in such studies could easily be confounded by additive genetic effects on a monogenic trait. This occurs if differences between the parents are small and there is difficulty in distinguishing this with the normally distributed population of a quantitative trait. Similar to this observation of continuous variation in the F_2, continuous variation of ODAP concentration in the present investigation together with normal distribution of the F_2 progenies indicated ODAP to be quantitatively inherited. Moreover, traits with continuous variation, high environmental influence and metric measurements are typical characteristics of a quantitative trait (A. Brule Babel, personal communication, 1993). Thus, contrary to the few reports on monogenic inheritance of ODAP, it was found to be inherited quantitatively in this study.

Heritability is an important tool to estimate the relative importance of genetics and the environment in phenotypic expression. It is the property of a particular situation and might not be applicable in other environments unless verified in several different environmental situations (A. Brule Babel, personal communication, 1993). Broad sense heritability estimates of ODAP in the present investigation varied from low to high. Estimated broad sense heritability ranged from 17% in the cross LS82046 x LS90235 to 93% in the cross L720060 x L900436. This very low to high broad sense heritability indicated that environmental influence on ODAP may be very large. However, it is the estimation of broad sense heritability which is not as reliable as an estimate of the fixable narrow sense heritability. Narrow sense heritability of the neurotoxin is reported to be in the range of 47% to 65% (Nerkar, 1972; Quader *et al.*, 1989).

One hundred and fifty species of the genus *Lathyrus* have been reported (Smart, 1990). A survey of the current literature suggested that ODAP or other toxin concentration in the genus *Lathyrus* have been reported only from 49 species (Table-5.1). Critical evaluation of the toxin concentration of rest of the species and attempts for interspecific hybridization would be highly recommended. However, interspecific hybridization in the genus *Lathyrus* have been reported to be unsuccessful in many instances (Baker, 1916; Senn, 1938; Davis, 1957; Smartt,

Table 5.1 Distribution of Lathyrogens in different species of *Lathyrus**

Lathyrogens	Species
2,4-diaminobutyric acid (Neurolathyrogen)	*L. aurantius* *L. luteus* *L. laevigatus*
2,3-Diaminobutyric acid, N-4-oxalyl-2,4-diaminobutyric acid and N-3-oxalyl-2,3-diaminopropionic acid (Neurolathyrogens)	*L. sylvestris* *L. latifolius* *L. heterophyllus* *L. gorgoni* *L. grandiflorus* *L. cirrhosus* *L. rotundifolius* *L. undulatus* *L. tuberosus* *L. multiflora*
N-3-oxalyl-2,3-diaminopropionic acid (ODAP, Neurolathyrogen)	*L. sativus* *L. setifolius* *L. alatus* *L. articulatus* *L. arvense* *L. pannonicus* *L. ochrus* *L. clymenum* *L. megallanicus* *L. quadrimarginatus* *L. cicera* *L. pseudocicera* *L. tremolsianus*
y-glutamyl-B-aminopropionitrile (Osteolathyrogen)	*L. odoratus* *L. hirsutas* *L. pusillus* *L. roseus*

* Compiled from Bell (1973)

In addition to the above, Lambein *et al.* (1986) reported following species to contain Lathyrogens:

L. amphicarpus, L. gmelinii, L. pannonicus, L. palustris, L. japonicus, L. laxiflorus, L. frolovii, L. angulatus, L. pratensis, L. venosus, L. ochroleucus, L. aphaca, L. clymenum, L. tingitanus, L. digitatus, L. inconspicuus, L. sphaericus, L. venetus, L. niger.

1984b; Khawaja, 1988; Murray and Hammat, 1989; Yunus and Jackson, 1991). Murray and Hammet (1989) attempted 6 different interspecific crosses between different species of *Lathyrus* and they were able to produce hybrid seed only from the cross of *L. chloranthus* and *L. chrysanthus* when the former was used as the female. However, the F_1 plants were all sterile. They postulated that, differences in the C banding pattern and irregularities of meiotic behaviour were the cause of hybrid sterility. Yunus and Jackson (1991) reported interspecific hybridization between grass pea and 15 wild species in section *Lathyrus* (There are 33 species in the section *Lathyrus*). Two species *L. cicera* and *L. amphicarpus* have been reported to produce viable seeds only when these wild species were utilized as the female parent. In a survey of *Lathyrus* species, Lambein *et al.* (1986) reported the presence of 14 secondary compounds including 9 isoxazolinones. Most of the species had a unique combination of these compounds. Since it has been shown by Simola (1967) that unequal non-protein amino acid pattern can provide a barrier against hybridization by inhibiting pollen tube growth, this may explain why inter-specific hybridization is very rare indeed. Pecket (1959) remarked that, *Lathyrus* species with the ability to hybridize, had identical or very similar composition of phenolic substances.

In characters like ODAP and protein content, considerable variation occurs in the results reported by different workers for the same crop and often in the same varieties. Many times, it is difficult to get reproducible results from the same samples. The situation might be more complicated when different parts of the seed contain very different chemical composition like ODAP (Prakash *et al.*, 1977). To what extent the variation reflects genuine difference among samples and to what extent it can be attributed to differences in methodology, environmental influence and experimental error is difficult to say. However, in the present study all the samples were analyzed by the same individual and the seed was well mixed before weighting for analysis. A sample of known ODAP concentration was used as an internal check in every lot of analysis. Moreover, this method (Campbell method) was found to be the most reproducible among various methods used around the world to determine ODAP concentration (M. Hussain, Personal communication, 1993).

Some aspects specific to the determination of seed characteristics would be justified to discuss here. The F_1 embryo is actually borne on the mother plant belonging to the parental generation while the seeds borne on the F_1 plant carry F_2 embryos. Reports on legumes suggest that the maternal genetic constitution by itself, along with filial constitution, determines the phenotype in respect of

characters such as, seed weight and protein content (Snoad and Arthur, 1974). Thus, the performance of the seeds harvested from F_1 plants will depend on the F_1 genotype, effect of filial generation, and possible cytoplasmic factors, even if we ignore environmental factors. The contribution of each source is unknown and can vary from one cross to another. However, in the present investigation, seed produced from a F_1 plant was taken as F_1 seed for analysis (which carried F_2 embryo) and seed produced by a F_2 plant was taken as F_2 seed (which actually carries F_3 embryo).

ODAP concentration of grass pea seed was also found to be affected by the stage of seed development in the pod. Relatively higher amounts of ODAP were detected from immature shrivelled seeds compared to plump seeds of the same plant (Figure 5.1). An intermediate level of ODAP was found in intermediate seed. This observation indicated that ODAP may be synthesized in the early stage of the developing pod and concentration may be diluted once the cotyledon is filled up or plumped with starch. Hence, it is important that analysis of ODAP be done on well matured seeds. Although both experimental years were fairly cool and wet when compared to normal years (Table 5.2 and 5.3) a considerable amount of seeds were not well matured at the time of harvesting therefore an effort was made to select only fully matured seeds for analysis in the present investigation.

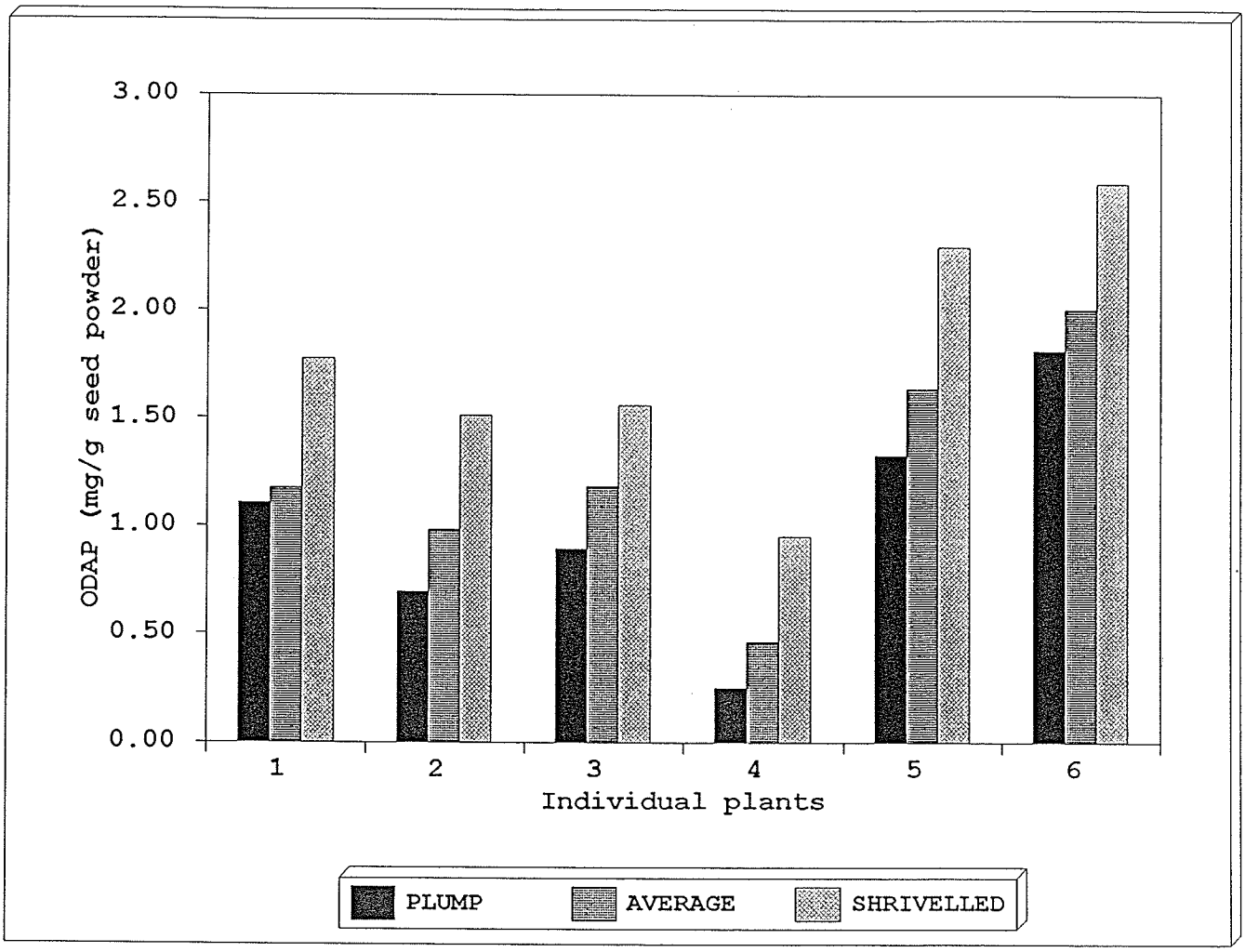


Figure 5.1 Comparison between plump and shrivelled seeds in seed ODAP concentration of grass pea.

Table 5.2 Monthly Weather Conditions Recorded During 1992 at Agriculture Canada Research Station, Morden, Manitoba.

	Mean Temp. °C				Precip. (mm)		
Month	Max.	Min.	Mean	LTA	1992	LTA	RD
April	7.7	-1.6	3.1	4.1	34.0	37.6	14
May	20.0	6.4	13.2	12.0	58.0	60.3	16
June	21.0	10.5	15.8	17.3	85.0	80.6	11
July	21.2	11.4	16.3	20.4	63.4	72.3	16
August	22.1	11.5	16.8	19.1	61.2	61.9	15
Sept.	18.0	5.9	11.9	12.9	35.2	52.0	11

LTA= Long term average of 74 years, RD= Number of rainy days.

Table 5.3 Monthly Weather Conditions Recorded During 1993 at Agriculture Canada Research Station, Morden, Manitoba.

	Mean Temp. °C				Precip. (mm)		
Month	Max.	Min.	Mean	LTA	1993	LTA	RD
April	10.6	-1.2	4.7	4.1	12.6	37.6	7
May	18.4	5.8	12.1	12.0	64.4	60.3	13
June	20.4	10.2	15.3	17.3	169.8	80.6	17
July	21.6	12.8	17.2	20.4	196.4	72.3	20
August	23.2	13.1	18.2	19.1	85.6	61.9	20
Sept.	17.1	5.5	11.3	12.9	17.6	52.0	13

LTA= Long term average of 74 years, RD=Number of rainy days.

Grass pea has been reported to be tolerant/resistant to both drought and flood along with other several environmental stresses (Kaul *et al.*, 1986; Lal *et al.*,1986; Dahiya, 1986; Bharati and Neupane, 1989; Basir, 1989; Smartt, 1990, 1994; Campbell *et al.*, 1994). A survey of the present literature did not provide any morphological or physiological explanation for this property. The relative resistance of grass pea to disease and insect attack has often been mentioned as a possible reason for the persisting popularity as an easily produced crop (Lambein, 1989). The resistance to insect attack seems to be unrelated to the concentration of ODAP present (Roy and Bhat, 1975) and its resistance to certain fungi seems to be unrelated to the seed's toxicity to man and animals (Lal *et al.*, 1986). In contrast, Swarp and Lal (1993) reported yield fluctuations from 645 kg/ha in 1985/86 to 299 kg/ha in 1987/88 largely due to moisture variations and differences in relative humidity. Dravid *et al.* (1985) found grass pea less tolerant to saline soil conditions when compared to pea (*Pisum sativum*) and gram (*Cicer arietinum*). They also reported that higher application rate of phosphorus was needed for grass pea as compared to pea. Thus, physiological and/or morphological studies are warranted in relation to hardiness of this neglected pulse crop.

6. SUMMARY AND CONCLUSION

Grass pea is an economically important food, feed and fodder legume crop particularly in the Indian sub-continent. It is known for its resistance to droughts and floods along with biotic and abiotic stresses. In extreme droughts and floods, a reasonably good yield of grass pea have been harvested when other crops completely failed, hence it is known as a survival food. The grains are used for human consumption in various preparations. Young plants are eaten as nutritious vegetables. The fodder component has always been very important for farmers since a reasonable yield can be expected even after grazing the field. The chaff and straw are fed to farm animals.

A strong epidemiological association is known to exist between consumption of grass pea and lathyrism. Neurolathyrism in humans has been reported to appear when grass pea forms a majority of the human diet for a extended period of time. This is a neurologic disease characterized by muscular rigidity, weakness, and paralysis of the leg muscle (spastic paraparesis). A neurotoxin, ß-N-Oxalyl-L-α,ß-diaminopropionic acid (ODAP) has been identified to be the causative principle. This neurotxin is present in all parts of plants. If the neurotoxin can be eliminated the crop could become a boon to the farmers of semi arid regions of the world. Wide variation

in ODAP concentration exists among grass pea lines though none of them are toxin free. This study was undertaken to investigate the mode of inheritance of the neurotoxin, a prerequisite for the development of neurotoxin free or very low neurotoxin lines.

Many of the progenies of low x low ODAP crosses were found to be low in ODAP concentration suggesting prevalence of similar genetic characters between low ODAP lines. However, wider variation in the F_2 progenies might indicate the presence of different modifier genes in the different lines. Cytoplasmic effect was detected in two of the low lines. Progenies of the line LS82046 produced higher seed ODAP concentration when this line was used as the female parent whereas progenies of the line L720060 segregated for low ODAP concentration when used as the female parent but not when used as the male parent. Distribution of the F_2 progenies was very close to normal with continuous variation suggesting that ODAP concentration was inherited quantitatively.

All the F_1 progenies of the crosses of low x high ODAP were intermediate in ODAP concentration indicating lack of complete dominance either for low or high ODAP concentration. Significantly higher ODAP was produced when the high ODAP line L880283 was used as the female parent in some of the low x high crosses, indicating prevalence of a cytoplasmic

influence. The F_2 progenies segregated covering the entire parental range. There was no distinct gap in the distribution of the segregating F_2 population, although the parents were well separated in seed ODAP concentration. The continuous variation, together with very close to normal distribution of the progenies indicated that ODAP was inherited quantitatively.

Broad sense heritability of ODAP concentration by comparing the segregating and homogeneous population was estimated to be in the range of 17 to 93%. This very low to high heritability estimates indicated that there could be a large environmental influence on ODAP concentration.

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8. APPENDIX

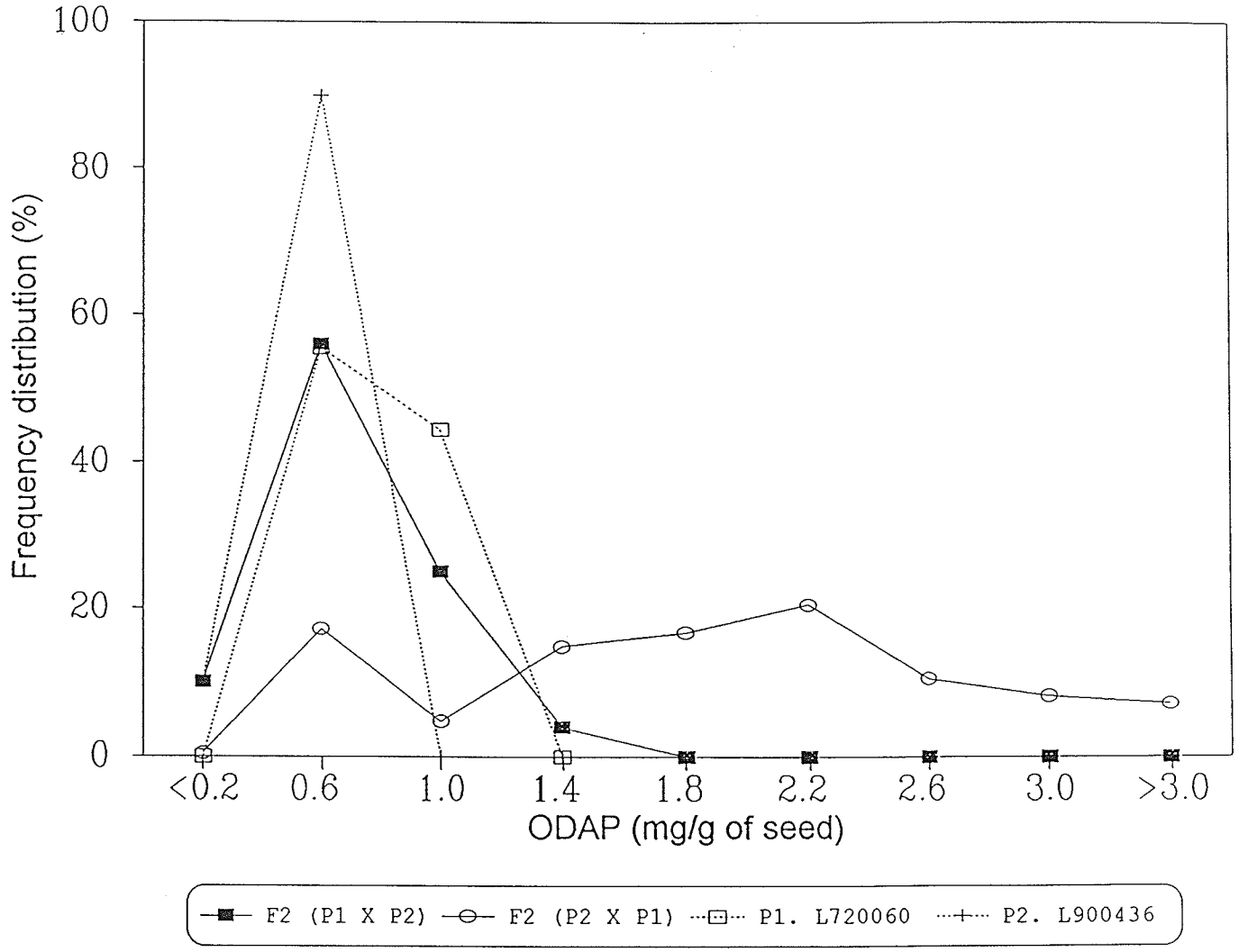


Figure 4.1.1 Distribution of ODAP concentration on Parents and F_2 progenies in the cross L720060 X L900436.

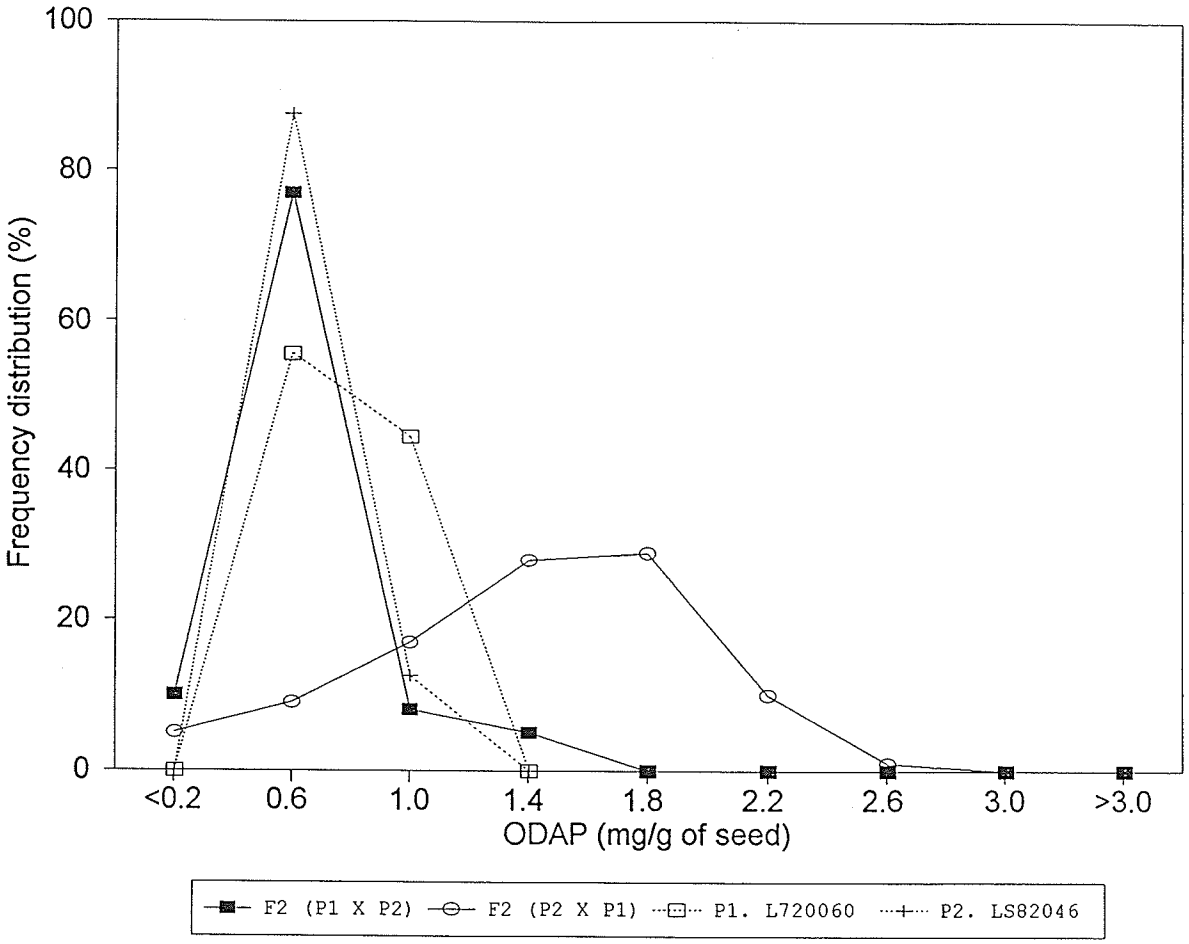
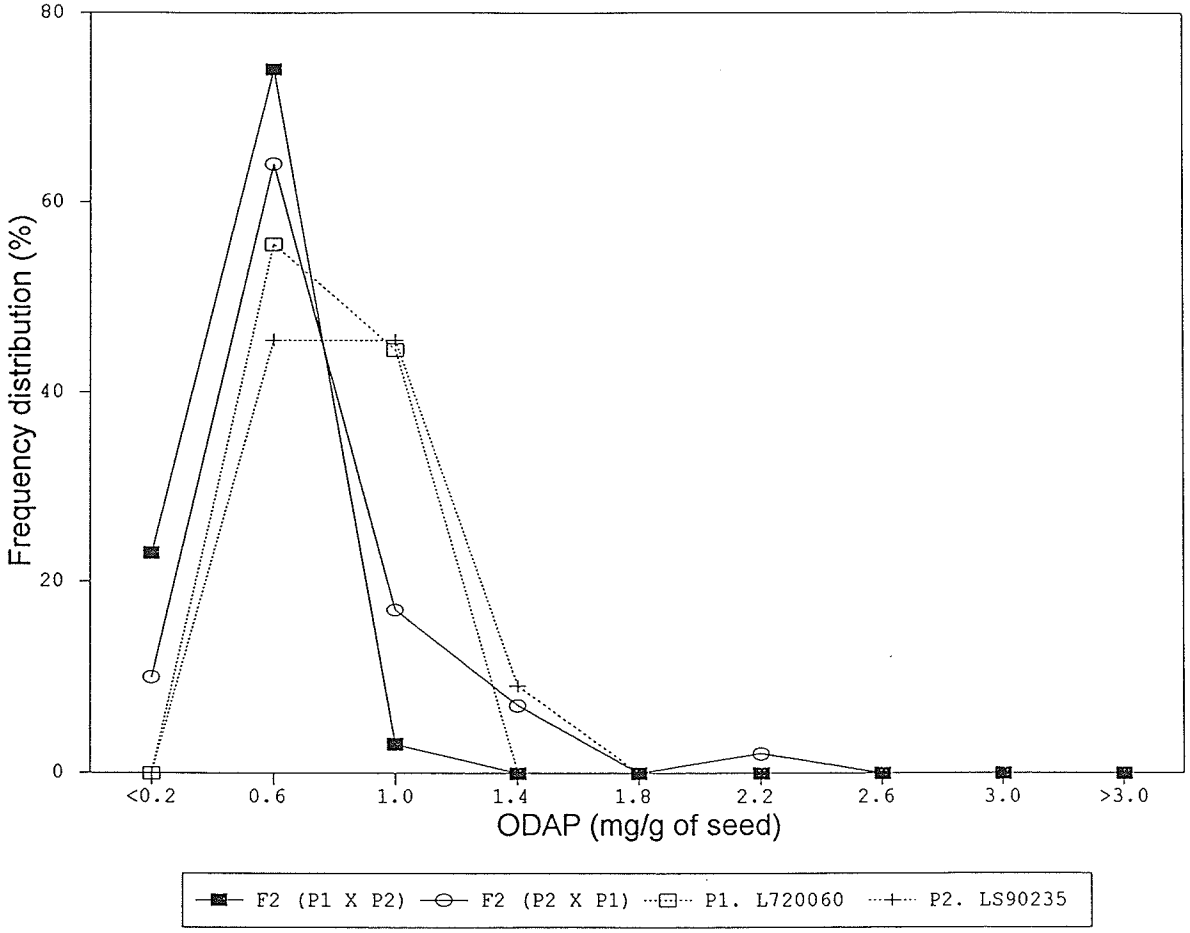


Figure 4.1.2 Distribution of ODAP concentration on Parents and F_2 progenies in the cross L720060 X LS82046.



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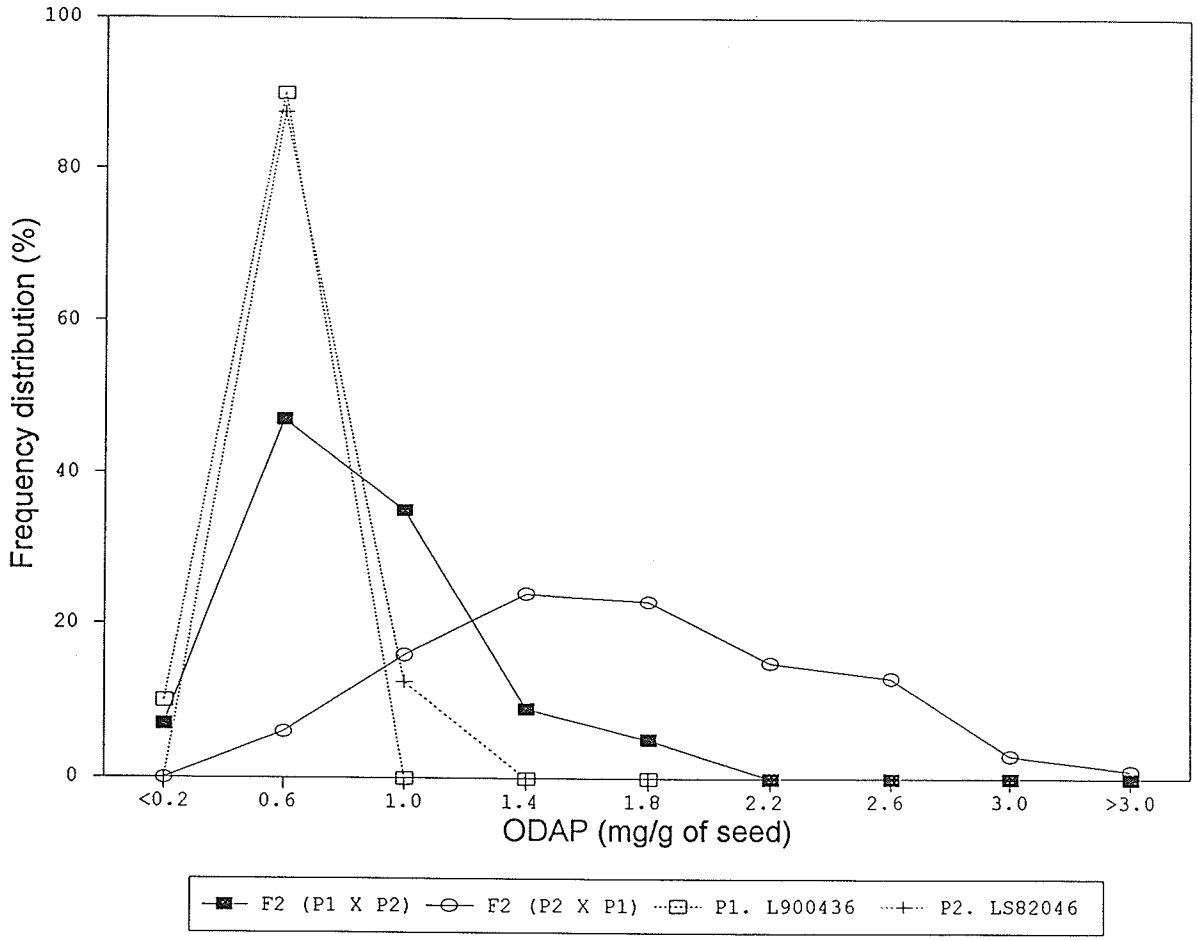

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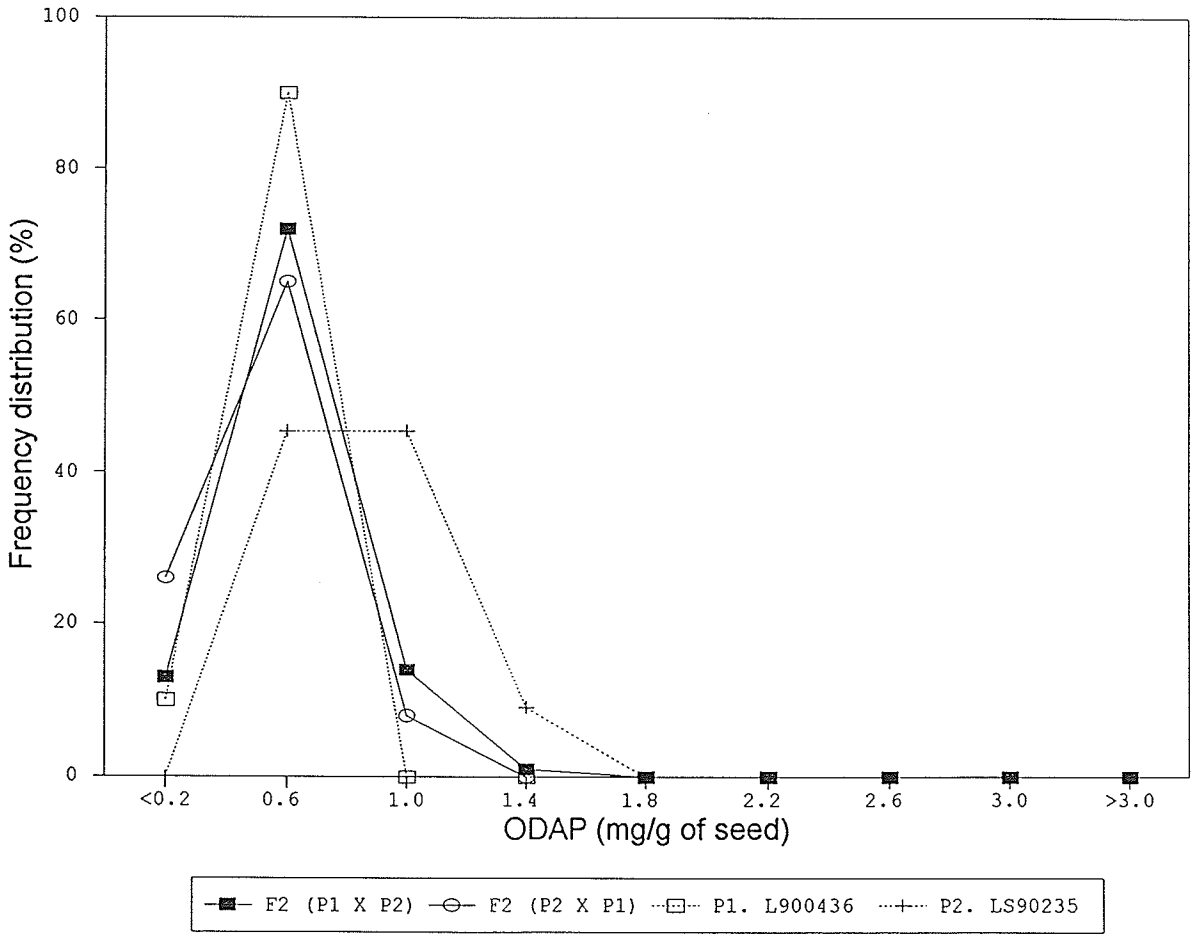


Figure 4.1.5 Distribution of ODAP concentration on Parents and F_2 progenies in the cross L900436 X LS90235.

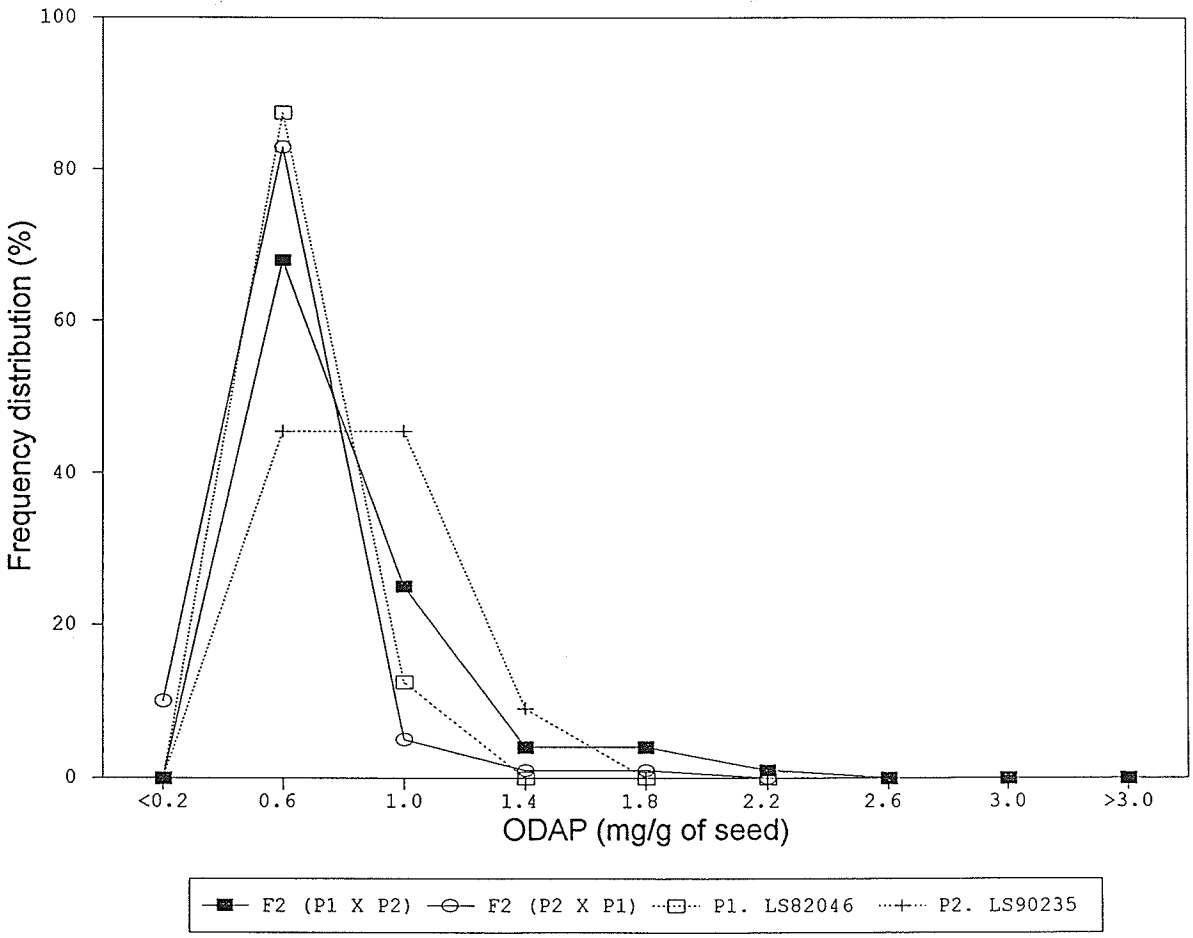
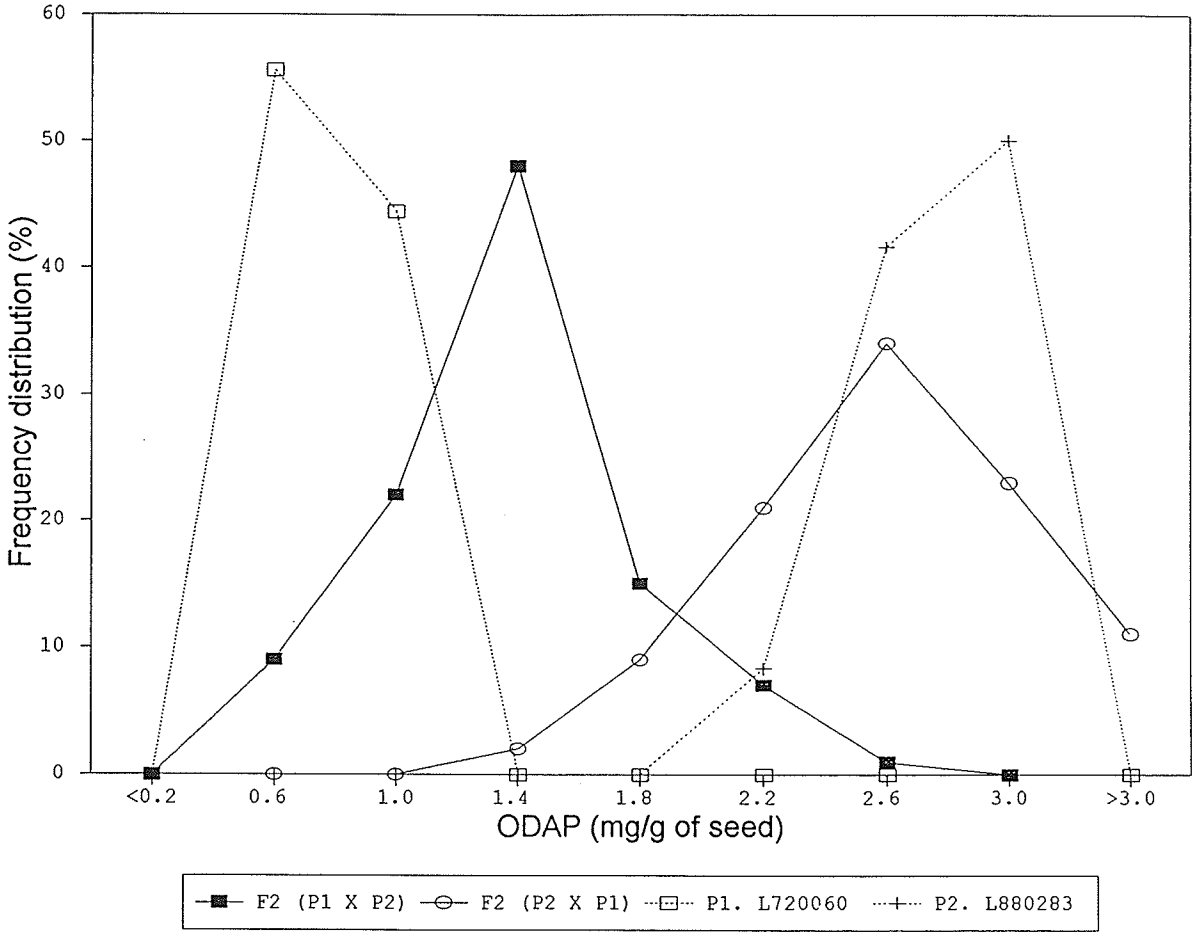


Figure 4.1.6 Distribution of ODAP concentration on Parents and F_2 progenies in the cross LS82046 X LS90235.



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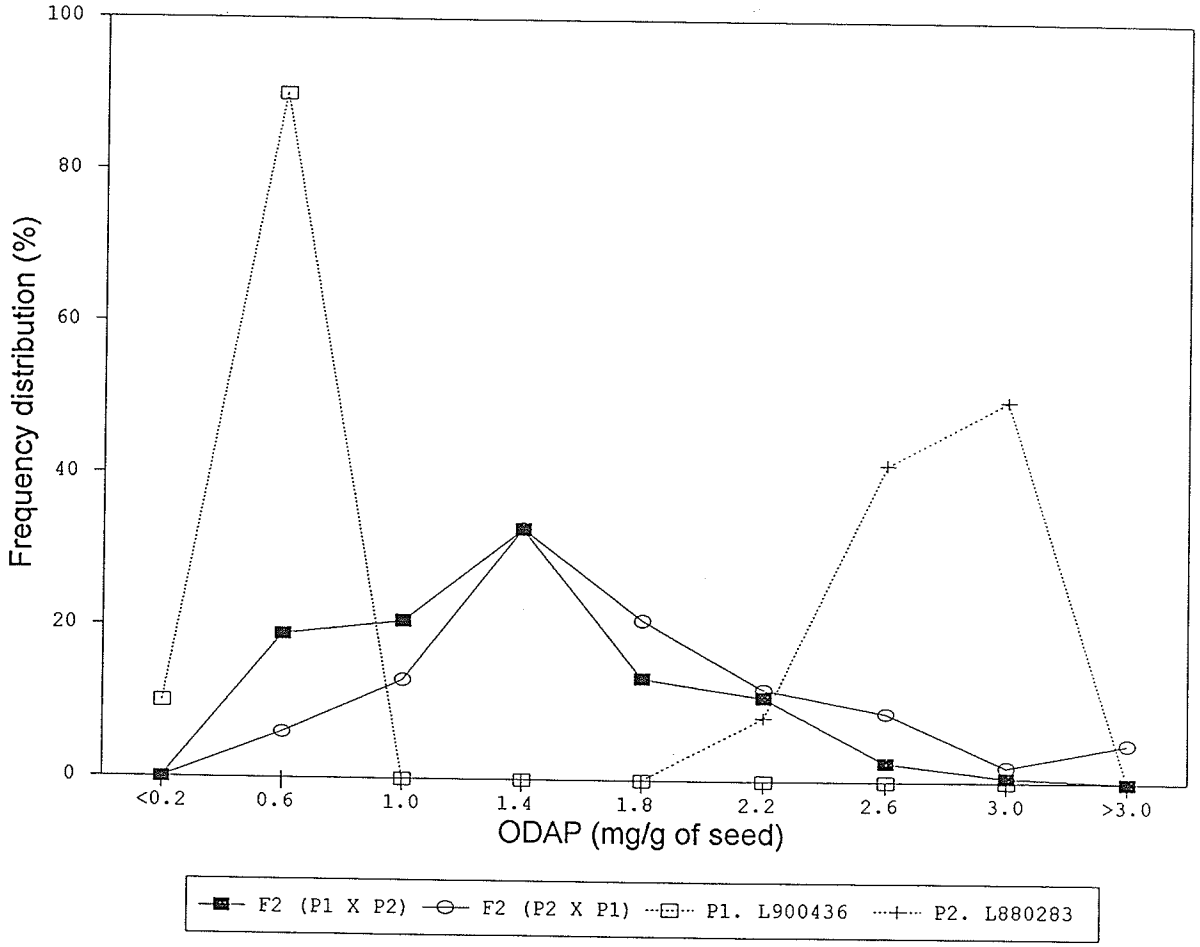

Figure 4.2.2 Distribution of ODAP concentration on Parents and F_2 progenies in the cross L900436 X L880283.

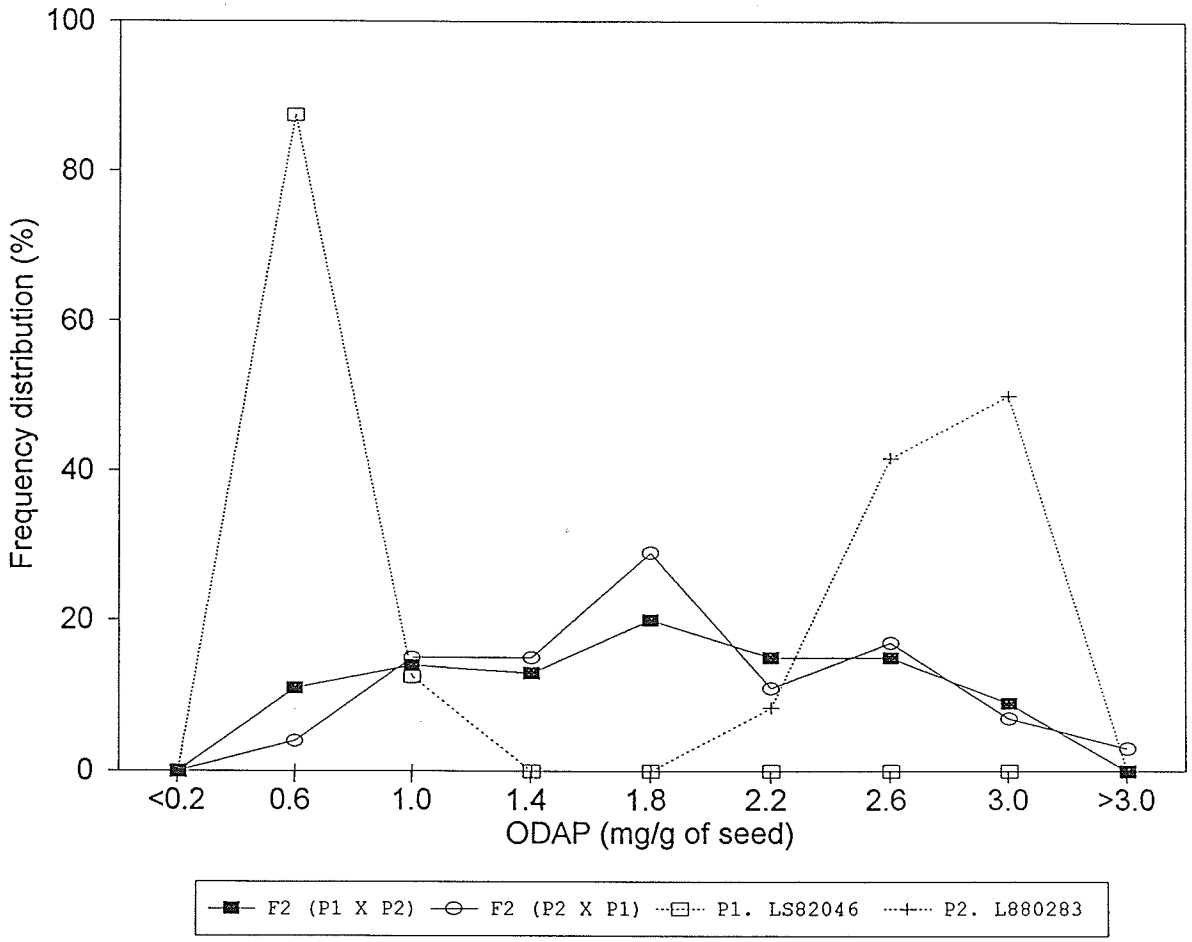

Figure 4.2.3 Distribution of ODAP concentration on Parents and F_2 progenies in the cross LS82046 X L880283.

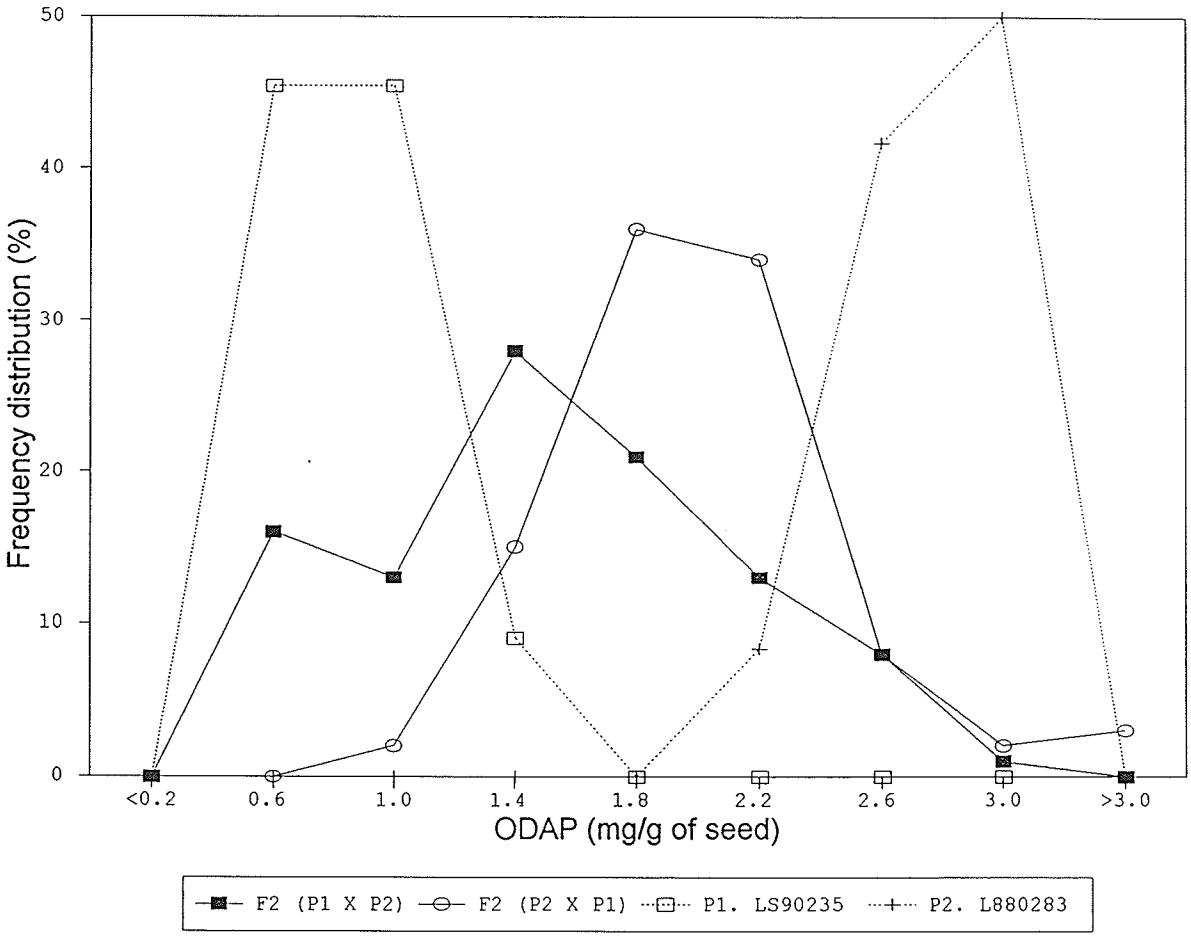

Figure 4.2.4 Distribution of ODAP concentration on Parents and F_2 progenies in the cross LS90235 X L880283.