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Oilseeds Crops: Agronomy, Science, and Technology

Guest Editors: Mohamed Fawzy Ramadan Hassanien, Sascha Rohn, Hesham Farouk Oraby, Bertrand Matthäus, and Abdalbasit Adam Mariod

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Editorial Board

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Editorial

Oilseeds Crops: Agronomy, Science, and Technology

Mohamed Fawzy Ramadan Hassanien,[1] Sascha Rohn,[2] Hesham Farouk Oraby,[3] Bertrand Matthäus,[4] and Abdalbasit Adam Mariod[5]

[1] *Department of Biochemistry, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt*
[2] *Institut für Lebensmittelchemie, Hamburg University, Grindelallee 117, 20146 Hamburg, Germany*
[3] *Cellulosic Biofuel Network, Soils and Crops Research and Development Center, Agriculture and Agri-Food Canada, Canada*
[4] *Institute for Lipid Research, Federal Research Center for Nutrition and Food, Germany*
[5] *Food Sciences and Technology Department, College of Agricultural Studies, Sudan University of Science & Technology, P.O. Box 71 Khartoum North, Sudan*

Correspondence should be addressed to Mohamed Fawzy Ramadan Hassanien, hassanienmohamed@yahoo.com

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Oilseeds are expected to play an increasing role in future food supply. Even though the species are diverse, interestingly there are many varieties which possess potentials for food production. Various seeds are generally rich in lipids and provide the major part of oil and fat needs of the populations.

Major conventional edible vegetables oils and fats produced worldwide are generally provided by cotton, soy, sunflower, rapeseed, peanut, palm kernel, and cocoa seeds using traditional and industrial processes. In many countries traditional processes for producing oil are very important, especially among rural communities which have easy access to raw oleaginous materials. Traditional processing tends to be environmentally sound and the skills required are family or group activities. The lack of suitable conservation methods for the local oilseeds before processing limits the opportunity for export. Food conservation constitutes a primordial problem in the tropics where postharvest losses are estimated at more than 50%. In large-scale production, refined oils are produced through development projects from raw materials like cottonseed using industrial operations. The edible oils obtained meet the requirements of urban consumers such as bland flavor and odor, clear appearance, light color, stability to oxidation, and suitability for frying.

While there are many uses for industrial vegetable oils, total world production is only about 3% of that of edible oils. Fats and oils are essential nutrients, comprising about 40% of the calories in the diet. Edible vegetable oils, margarine, and shortening are used food applications. These products supplement or replace animal products (e.g., butter and lard), supplies of which are inadequate to meet the needs of an increasing world population. On the other side, oilseed meals are rich in protein; mixed with other ingredients (e.g., cereal grains), they provide nutritionally balanced feeds.

Traditionally, the main non-food uses for fats and oils have been in the manufacture of soaps and detergents and in the production of greases, lubricants, and candles. More recently, the biofuels market has provided significant new, non-food use for edible oilseeds; it is used as the feedstock for the production of biodiesel and as an alternative to mineral oils for agricultural machinery. However, edible oilseeds are not in surplus supply and such raw materials for biodiesel are not useful from an economical point of view. Therefore, Jatropha curcas characterized by a rapid growth, low cost of seeds, and high oil content (ca. 50%) has been successfully tested for biodiesel production.

Fats and oils as sources of carbon-carbon double bonds can undergo transformation by metathesis reaction to form intermediates, which could then be used for the synthesis of a wide range of reaction products ranging from pharmaceuticals and cosmetics to polymers and fine chemicals. In many countries, hectares of lands available as well as a favorable climate for the production of oil crops and for stock farming. These must be considered as new opportunities for the continent that is currently positioning itself to take a more proactive role in the global economy.

Although a lot of literature is available on the physicochemical and nutritional characteristics of conventional vegetable oils and fats, very few researches based on nonconventional oil seeds for industry are made. In this special issue concerning oilseeds crops, international researchers contributed original research papers and review articles on potential topics including crop management, genetics and breeding, genomics and biotechnology, plant protection, quality and nutrition of seed oils, biofuels, and economics.

Acknowledgment

Special thanks are due to guest editors Professor Rohn Sascha, Dr. H. F. Oraby, Dr. B. Matthäus, and Dr. A. A. Mariod for efforts been made to review and edit the articles for this special issue.

Mohamed Fawzy Ramadan Hassanien
Rohn Sascha
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Research Article

Effect of Pot Size on Various Characteristics Related to Photosynthetic Matter Production in Soybean Plants

Minobu Kasai, Keisuke Koide, and Yuya Ichikawa

Department of Biology, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan

Correspondence should be addressed to Minobu Kasai, minobu@cc.hirosaki-u.ac.jp

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Despite the wide uses of potted plants, information on how pot size affects plant photosynthetic matter production is still considerably limited. This study investigated with soybean plants how transplantation into larger pots affects various characteristics related to photosynthetic matter production. The transplantation was analyzed to increase leaf photosynthetic rate, transpiration rate, and stomatal conductance without affecting significantly leaf intercellular CO_2 concentration, implicating that the transplantation induced equal increases in the rate of CO_2 diffusion via leaf stomata and the rate of CO_2 fixation in leaf photosynthetic cells. Analyses of Rubisco activity and contents of a substrate (ribulose-1,5-bisphosphate (RuBP)) for Rubisco and total protein in leaf suggested that an increase in leaf Rubisco activity, which is likely to result from an increase in leaf Rubisco content, could contribute to the transplantation-induced increase in leaf photosynthetic rate. Analyses of leaf major photosynthetic carbohydrates and dry weights of source and sink organs revealed that transplantation increased plant sink capacity that uses leaf starch, inducing a decrease in leaf starch content and an increase in whole plant growth, particularly, growth of sink organs. Previously, in the same soybean species, it was demonstrated that negative correlation exists between leaf starch content and photosynthetic rate and that accumulation of starch in leaf decreases the rate of CO_2 diffusion within leaf. Thus, it was suggested that the transplantation-induced increase in plant sink capacity decreasing leaf starch content could cause the transplantation-induced increase in leaf photosynthetic rate by inducing an increase in the rate of CO_2 diffusion within leaf and thereby substantiating an increase in leaf Rubisco activity in vivo. It was therefore concluded that transplantation of soybean plants into larger pots attempted in this study increased the plant photosynthetic matter production by increasing mainly sink capacity that uses leaf starch for whole plant growth, particularly, growth of sink organs.

1. Introduction

Plant photosynthetic matter production is affected by various environments. In studies for understanding how plant photosynthetic matter production responds to various environments and what mechanisms are responsible for the responses, there are cases that potted plants are used. There are also cases that potted plants are dealt with as commercial goods or foods. It is important to accumulate information of how photosynthetic matter production in potted plants is affected by pot size, since even in the future potted plants will be used by many people for various uses. However, the information has been only a few, and only in recent years the importance of pot size for plant photosynthetic matter production was shown with scientific data by Arp [1]. The author collected data of pot size and data related to photosynthetic matter production from a number of studies that had conducted high CO_2 treatment experiments in potted plants, and reported that there were roughly positive correlations between pot size and leaf photosynthetic rate and pot size and increased ratio of root to shoot and pot size and leaf chlorophyll content [1]. The high CO_2 treatment experiments have been conducted to examine responses of plants to high CO_2 environments that will come in the future [2]. Arp pointed out from collected data and his own research data [3] that downregulation of leaf photosynthesis can occur more in potted plants than in field grown plants [1], and with respect to the reason(s), he pointed out the importance of a well-known hypothesis that there is downregulation of leaf photosynthesis through

accumulation of photosynthetic carbohydrate in leaf, which occurs from photosynthetic source capacity that is excessive to sink capacity of sink organs such as roots, although the detailed mechanism(s) is still unclear [1]. To our knowledge, since Arp, only one study using cotton seedlings provided information that smaller pots decreased leaf photosynthetic rate and stomatal conductance and increased leaf starch content [4].

In point of photosynthetic source-sink balance, for example, growing plant materials with smaller pots may be similar to removing sink organs (e.g., flowers, fruits, or pods) from plant materials. A number of studies have used the manipulation that removes sink organs from plant materials to examine how reducing plant sink capacity affects photosynthetic matter production [5, 6]. However, removal of sink organs from plant materials is not identical to growing plant materials with smaller pots. Smaller pots should affect in particular sink organs of roots, since roots are mainly present within the pots. In addition, the removal of sink organs gives excisions' damage to plant materials [6]. Thus, to obtain more information of how pot size affects plant photosynthetic matter production, it is important to alter pot size directly. This study investigated the effect of altering pot size on plant photosynthetic matter production using soybean, which is one of the most important crops grown in the world [7, 8]. Actually, with potted soybean plants, it was analyzed how transplantation into larger pots affects various characteristics related to photosynthetic matter production, that is, leaf photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration, initial and total activities of Rubisco in leaf extract, contents of a substrate (RuBP) for Rubisco, major photosynthetic carbohydrates (sucrose and starch), total protein and chlorophyll in leaf, and dry weights of source and sink organs. In similar studies other than this study, the same series of analyses have not been conducted, and transpiration rate and content of total protein in leaf and initial and total activities of Rubisco in leaf extract have not been analyzed.

2. Materials and Methods

Soybean (*Glycine max* L. Merr. cv. Tsurunoko) seeds were sown in plastic pots (11.4 cm in height, 7.5 cm in diameter) containing mixed vermiculite and sand (1 : 1 in volume) and grown in growth chambers (Koitotoron, HNL type; Koito Industries Ltd., Tokyo, Japan) under daily light/dark periods of 10/14 h, day/night temperatures of 24/17°C, and relative humidity of 60%. After 40 days, half of the plants were transplanted into larger pots (24 cm in height, 20 cm in diameter) and grown with the remaining plants (controls) for 14 or 24 days under the same growth conditions. Nutrients were supplied twice a week with a 1000-fold diluted solution of Hyponex (6-10-5 type (N : P : K = 6 : 10 : 5); Hyponex Co., Osaka, Japan), and tap water was supplied in sufficient amounts. Intensity of light, which was supplied with incandescent lamps, was 80 μmol photons $m^{-2}\,s^{-1}$ (400–700 nm) on top of the original pot.

Leaf photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentration were determined in fully expanded middle trifoliate leaves mainly on day 14 and 24 after transplantation at a light intensity of 800 μmol photons $m^{-2}\,s^{-1}$, air flow rate of 200 mL min^{-1}, air temperature of 25°C, relative humidity of 60%, and CO_2 concentration of 350 ppm using a portable photosynthetic analyzer (Cylus-1; Koito Industries Ltd.). After measurements, leaf disks (1.79 cm^2) were taken from the middle trifoliate leaves for the other analyses, as described previously [5].

The initial and total activities of Rubisco in leaf extract were determined at 25°C as described previously [5]. Content of RuBP in leaf was determined as described below. To a leaf extract obtained by homogenizing a leaf disk with an ice-cold buffer (100 mM HEPES-KOH, pH 7.8, 1 mL), $HClO_4$ (final conc., 0.5 M) was added, and the mixture was centrifuged (10,000 g, 10 min) after leaving on ice for 10 min. The resulting supernatant was centrifuged (10,000 g, 10 min) after neutralizing to pH 5.6 with K_2CO_3, and the supernatant was used for the determination of RuBP content [5]. The content of total protein in leaf was determined by quantifying protein included in leaf extract that had been prepared for determination of Rubisco activity by the method of Bradford [9]. The leaf chlorophyll content was determined according to the method of Mackinney [10]. The contents of sucrose and starch in leaf were determined as described by Sawada et al. [11]. Dry weights of source (leaves) and sink organs (stems, floral organs including pods, and roots) were determined for plants on day 24 after transplantation. Each organ was dried at 75°C for a week.

3. Results

On both days 14 and 24 after transplantation, the analyzed leaf photosynthetic rate and transpiration rate were significantly higher in transplanted soybean plants than in control plants (Figure 1). Leaf stomatal conductance was also higher in transplanted plants than in control plants on both days, while leaf intercellular CO_2 concentration did not differ significantly between control and transplanted plants (Figure 2). Initial and total activities of Rubisco in leaf extract were significantly higher in transplanted plants than in control plants on both days (Figure 3), while the activation ratios (initial activity/total activity) did not differ significantly between control and transplanted plants (not shown). Contents of chlorophyll and total protein in leaf were significantly higher in transplanted plants than in control plants on both days (Figure 4). Leaf RuBP content was significantly (day 14) or on the average lower (day 24) in transplanted plants than in control plants (Figure 5). Leaf sucrose content was significantly higher in transplanted plants than in control plants, while leaf starch content was significantly lower in transplanted plants than in control plants on both days (Figure 6). Dry weights of leaves, floral organs including pods, and roots in transplanted plants on day 24 were significantly heavier than those in control plants, while dry weight of stems did not differ significantly between

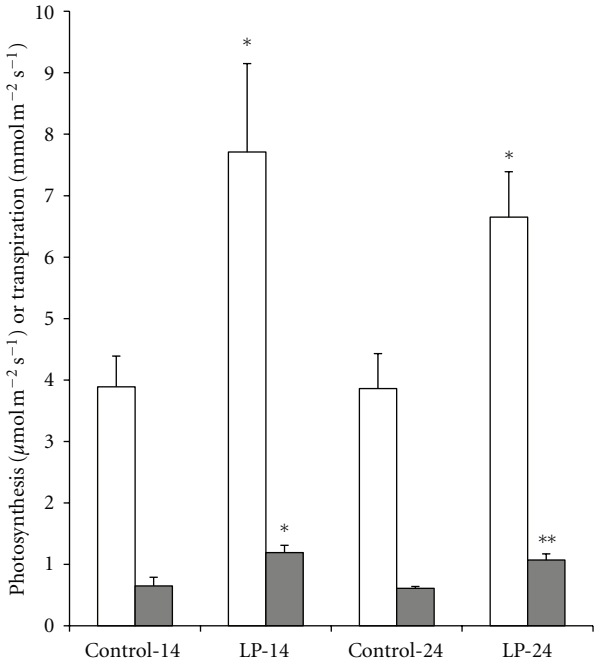

Figure 1: Leaf photosynthetic rate and transpiration rate in soybean plants grown with original pots (Control) and with larger pots (LP). Half of plants with age of 40 days were grown with larger pots for 14 (Control-14 or LP-14) or 24 days (Control-24 or LP-24). Open bar, photosynthetic rate; closed bar, transpiration rate. Vertical bars, S.D. ($n = 3$). $^{*}P < 0.05$/ $^{**}P < 0.01$ (t-test) when compared with control.

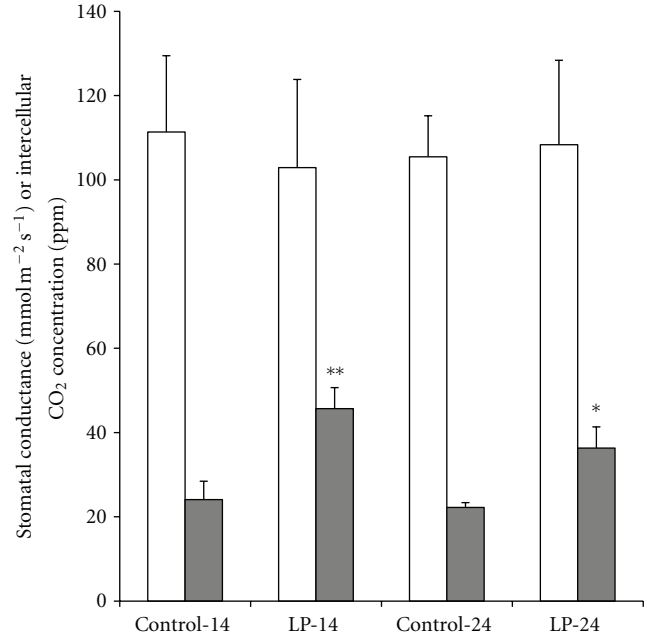

Figure 2: Leaf stomatal conductance and intercellular CO_2 concentration in soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Open bar, intercellular CO_2 concentration; closed bar, stomatal conductance. Vertical bars, S.D. ($n = 3$). $^{*}P < 0.05$/ $^{**}P < 0.01$ (t-test) when compared with control.

control and transplanted plants (Figure 7). When the ratio of sink (stems + floral organs + roots) to source organs (leaves) was calculated, those in control and transplanted plants were on the average 2.26 (100%) and 2.60 (115%), respectively. Transplantation did not affect dry weight of stems. The ratios in control and transplanted plants calculated without stems were on the average 1.66 (100%) and 2.13 (128%), respectively.

4. Discussion

To obtain more information concerning the effect of pot size on photosynthetic matter production in potted plants, this study investigated how transplantation of soybean plants into larger pots affects various characteristics related to photosynthetic matter production. The characteristics were analyzed mainly on day 14 and 24 after transplantation in control and transplanted plants. It was shown that leaf photosynthetic rate, transpiration rate, and stomatal conductance were higher in transplanted plants than in control plants on both days (Figures 1 and 2). As leaf photosynthetic rate, transpiration rate, and stomatal conductance in control plants that were measured just before transplantation did not differ significantly from those measured on day 14 and 24 after transplantation (not shown), these results indicate that the transplantation increased leaf photosynthetic rate, transpiration rate and stomatal conductance and strongly suggest that the transplantation increased the rate of CO_2 diffusion via leaf stomata. It is speculated that transplantation might have increased leaf photosynthetic rate through an increase in the rate of CO_2 diffusion via leaf stomata. However, data of Figure 2 indicate that transplantation did not affect significantly leaf intercellular CO_2 concentration, implicating that transplantation increased equally the rate of CO_2 diffusion via leaf stomata and the rate of CO_2 fixation in leaf photosynthetic cells.

As shown in Figures 3 and 4, initial and total activities of Rubisco in leaf extract and leaf total protein content were higher in transplanted plants than in control plants on both days. When the ratio of Rubisco activity (initial or total activity) and the ratio of leaf total protein content of transplanted plants relative to control plants were calculated from mean values of data, on both days the former and latter ratios were roughly consistent. The former and latter ratios were also roughly consistent with the ratio of leaf photosynthetic rate of transplanted plants relative to control plants calculated from mean values of data on both days. The ratios on days 14 and 24 were 2.6 and 2.6, respectively, for initial activity of Rubisco, and 3.0 and 2.8, respectively, for total activity of Rubisco, and 1.9 and 2.3, respectively, for leaf total protein content, and 2.0 and 1.7, respectively, for leaf photosynthetic rate. These results strongly suggest that transplantation increased leaf Rubisco activity in vivo and suggest that the increase in leaf Rubisco activity, which is likely to result from an increase in leaf Rubisco content, could contribute to the transplantation-induced increase in

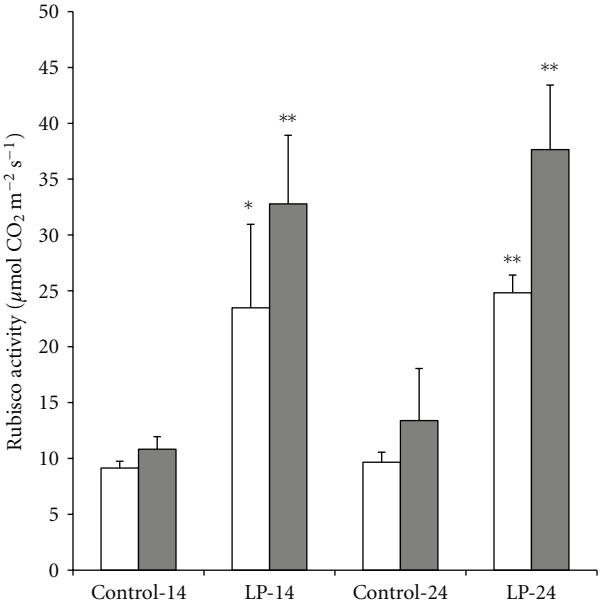

Figure 3: Initial and total activities of Rubisco in leaf extract from soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Open bar, initial activity; closed bar, total activity. Vertical bars, S.D. ($n = 3$). $^{*}P < 0.05$/$^{**}P < 0.01$ (t-test) when compared with control.

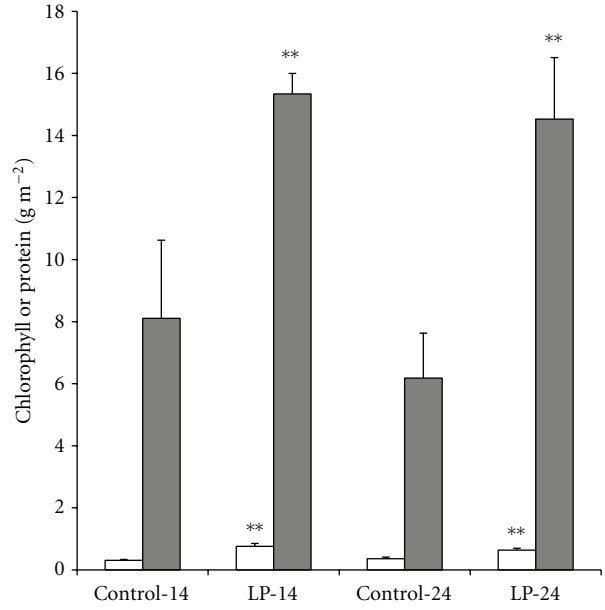

Figure 4: Leaf chlorophyll and total protein contents in soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Open bar, chlorophyll; closed bar, total protein. Vertical bars, S.D. ($n = 3$). $^{**}P < 0.01$ (t-test) when compared with control.

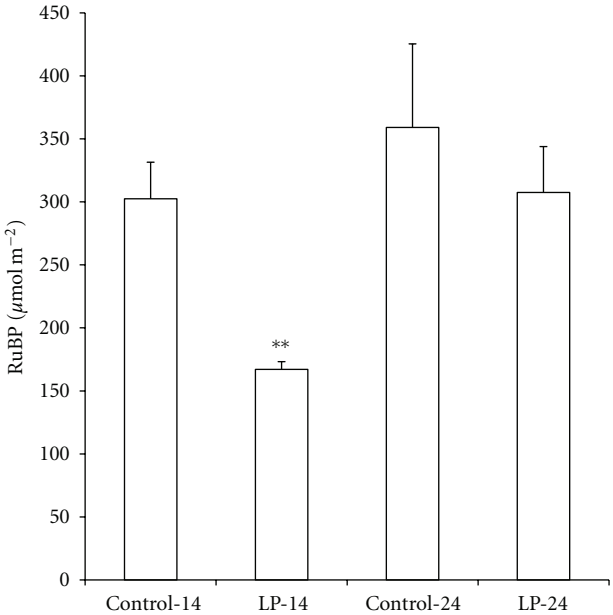

Figure 5: Leaf RuBP content in soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Vertical bars, S.D. ($n = 3$). $^{**}P < 0.01$ (t-test) when compared with control.

leaf photosynthetic rate. It is well known that in plants, Rubisco is a considerably major protein in leaf [12, 13]. There is evidence from studies altering expressions of Rubisco or its activation enzyme, Rubisco activase that changes in the activity and/or the amount of Rubisco in leaf significantly affect leaf photosynthetic rate [12, 13].

Data of Figure 4 indicate that transplantation increased leaf chlorophyll content. Arp found that a rough and positive correlation exists between pot size and leaf photosynthetic rate or pot size and leaf chlorophyll content [1]. The findings by Arp implicate that a rough and positive correlation may exist between leaf chlorophyll content and photosynthetic rate. Our results support the findings by Arp and the implication. Thus, an increase in leaf chlorophyll content might have also contributed to the transplantation-induced increase in leaf photosynthetic rate. However, there is a report that chlorophyll-less soybean isolines had similar leaf photosynthetic rate as the wild type at full sun photosynthetic photon flux densities [14].

RuBP is a substrate for Rubisco. Thus, it is thought that leaf RuBP content decreases when leaf Rubisco activity increases. Leaf RuBP content in transplanted plants was significantly or on the average lower than that in control plants (Figure 5). This result supports the suggestion that transplantation increased leaf Rubisco activity in vivo. In single-rooted soybean leaves that are the same species as we used in this study, it was demonstrated that continuous exposure to light, which increases photosynthetic source capacity, or treatment of roots with low temperatures, which decreases root sink capacity, decreases the leaf photosynthetic rate and Rubisco activity and increases the leaf RuBP content [15–18].

Sucrose and starch are the major photosynthetic carbohydrates in plants. In this study, it was shown that whereas leaf sucrose content was higher in transplanted plants than in control plants, leaf starch content was lower in transplanted

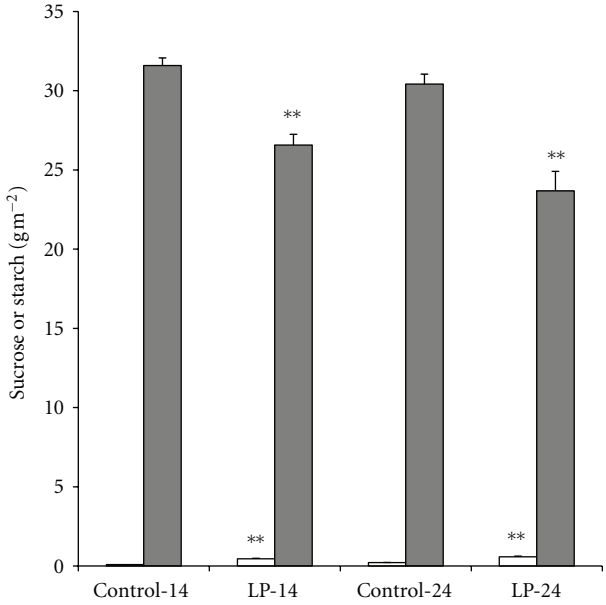

Figure 6: Leaf sucrose and starch contents in soybean plants grown with original pots (Control) and with larger pots (LP). Summary of growth conditions is described in Figure 1. Open bar, sucrose; closed bar, starch. Vertical bars, S.D. ($n = 3$). $^{**}P < 0.01$ (t-test) when compared with control.

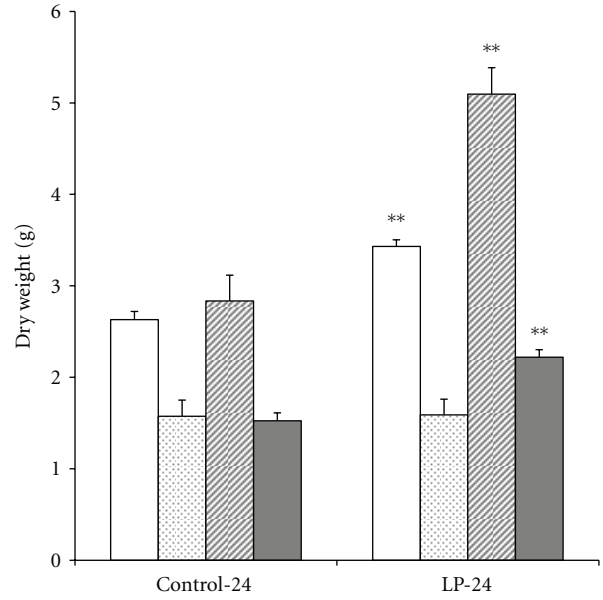

Figure 7: Dry weights of source (leaves) and sink organs (stems, floral organs or roots) in soybean plants grown with original pots (Control) and with larger pots (LP). Half of plants with age of 40 days were grown with larger pots for 24 days (Control-24 or LP-24). Open bar, leaves; dotted bar, stems; shaded bar, floral organs; closed bar, roots. Vertical bars, S.D. ($n = 3$). $^{**}P < 0.01$ (t-test) when compared with control.

plants than in control plants on both days (Figure 6). However, when the total content of sucrose and starch in leaf was calculated, the content was lower in transplanted plants than in control plants on both days (not shown). As data of Figure 7 indicate that transplantation increased dry weights of source and sink organs and the ratio of sink to source organs, these results indicate that transplantation increased the plant sink capacity that uses especially leaf starch for whole plant growth, particularly, growth of sink organs.

There is a hypothesis of inhibition of photosynthesis through accumulation of sucrose in leaf, although the regulatory mechanism(s) is still unclear [5]. For example, in a study conducting continuous exposure to light of single-rooted soybean leaves, it was demonstrated that significant negative correlation exists between leaf sucrose content and photosynthetic rate [15]. In this study, leaf sucrose content was higher in transplanted plants that had higher leaf photosynthetic rate than in control plants on both days (Figures 1 and 6). It is thought that in transplanted plants, sucrose-induced inhibition of leaf photosynthetic rate was, if any, very small. Leaf sucrose content of transplanted plants on day 24, which was the highest content observed in this study, was about one third of a content that led to a small decrease (about 25%) in leaf photosynthetic rate of single-rooted soybean leaves [15]. There is also a hypothesis of inhibition of photosynthesis through accumulation of starch in leaf [5]. In the same study using single-rooted soybean leaves, it was also demonstrated that significant negative correlation exists between leaf starch content and photosynthetic rate [15]. Another study using single-rooted soybean leaves demonstrated that accumulation of starch in leaf decreases the rate of CO_2 diffusion within leaf [19]. Leaf starch content of control plants on day 14, which was the highest content observed in this study, almost corresponded with a content that led to a large decrease (about 45%) in leaf photosynthetic rate of single-rooted soybean leaves [15]. Data of Figure 6 indicate that transplantation decreased leaf starch content. Therefore, it is suggested that the decrease in leaf starch content could cause the transplantation-induced increase in leaf photosynthetic rate by inducing an increase in the rate of CO_2 diffusion within leaf and thereby substantiating an increase in leaf Rubisco activity in vivo. As transplantation increased plant sink capacity that uses leaf starch for whole plant growth, particularly, growth of sink organs, it is concluded from data of this study that transplantation of soybean plants into larger pots attempted in this study increased the plant photosynthetic matter production by increasing mainly sink capacity that uses leaf starch for whole plant growth, particularly, growth of sink organs. Although it is not study investigating the effect of pot size on plant photosynthetic matter production, a number of studies have implicated that there is downregulation of leaf photosynthesis through accumulation of photosynthetic carbohydrate (sucrose and/or starch) in leaf [5].

As described in the Introduction, a study using cotton seedlings showed that smaller pots decreased leaf photosynthetic rate and stomatal conductance and increased leaf starch content [4]. Our results are consistent with the report.

The study, however, did not analyze activity of Rubisco in leaf extract and contents of total protein, chlorophyll and RuBP in leaf. To our knowledge, until now, in similar studies other than this study that have examined the effect of pot size, a series of analyses we carried out have not been conducted, and transpiration rate and content of total protein in leaf and initial and total activities of Rubisco in leaf extract have not been analyzed.

Arp found from collected data that a rough and positive correlation exists between pot size and leaf photosynthetic rate or pot size and increased ratio of root to shoot [1]. The findings by Arp implicate that a rough and positive correlation may exist between leaf photosynthetic rate and increased ratio of root to shoot. Our results essentially support the implication, since transplantation increased leaf photosynthetic rate and the dry weight ratio of sink to source organs (Figure 7). Since the findings by Arp [1] and the study using cotton seedlings [4], substantial information on the effect of pot size on plant photosynthetic matter production has been scarce.

Regarding the phenomena seen in soybean plants in this study, essential similarity has also been seen in plants subjected to other manipulations to alter source or sink capacity. For example, removal of developing pods (soybean plants), which decreases sink capacity, was shown to result in accumulation of major photosynthetic carbohydrate (sucrose) in leaf, decrease in leaf photosynthetic rate, and decrease in Rubisco activity of leaf extract [20]. Data from other studies conducting removal of floral organs or petiole girdling, which decreases sink capacity, or continuous exposure to light, which reduces sink capacity by increasing photosynthetic source capacity, suggest that a decrease in stomatal conductance or Rubisco activity or Rubisco content in leaf, or both decreases in Rubisco activity and Rubisco content in leaf are responsible for the reduced sink capacity-induced decrease in leaf photosynthetic rate [21–28]. In potato and *Arabidopsis*, continuous exposure to light has been shown to accelerate expressions of photosynthetic genes, pigments and proteins, and subsequent declines of the expressions [29, 30]. It is important to elucidate the detailed mechanism(s) of how pot size affects plant photosynthetic matter production. Some studies have described mechanism(s) concerning regulation of plant leaf photosynthesis through photosynthetic source-sink balance, although they are not studies that have investigated the effect of pot size on plant photosynthetic matter production. For example, studies using transgenic plants suggest that hexokinase may be involved in carbohydrate-mediated repression of photosynthetic gene expression [31–33]. Other study shows that protein kinases (KIN10 and KIN11) may be involved in governing the entirety of carbohydrate metabolism, growth, and development in response to carbohydrates in plants [34]. However, the precise mechanism of how hexokinase and protein kinases exercise regulation of photosynthetic carbohydrate metabolism including the carbohydrate-mediated repression of photosynthetic gene expression is still unclear. Data obtained in this study and those from other studies that have investigated the effect of pot size on plant photosynthetic matter production strongly suggest that pot size can largely affect plant leaf photosynthesis and organization of source and sink organs, including the capacities of source and sink. Therefore, further studies are important for elucidation of the mechanism(s) responsible for regulation of plant photosynthetic matter production through photosynthetic source-sink balance.

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Review Article

Advances in Agronomic Management of Indian Mustard (*Brassica juncea* (L.) Czernj. Cosson): An Overview

Kapila Shekhawat, S. S. Rathore, O. P. Premi, B. K. Kandpal, and J. S. Chauhan

Directorate of Rapeseed-Mustard Research, Sewar, Rajasthan Bharatpur 321 303, India

Correspondence should be addressed to Kapila Shekhawat, drrathorekapila@gmail.com

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India is the fourth largest oilseed economy in the world. Among the seven edible oilseeds cultivated in India, rapeseed-mustard contributes 28.6% in the total oilseeds production and ranks second after groundnut sharing 27.8% in the India's oilseed economy. The mustard growing areas in India are experiencing the vast diversity in the agro climatic conditions and different species of rapeseed-mustard are grown in some or other part of the country. Under marginal resource situation, cultivation of rapeseed-mustard becomes less remunerative to the farmers. This results in a big gap between requirement and production of mustard in India. Therefore site-specific nutrient management through soil-test recommendation based should be adopted to improve upon the existing yield levels obtained at farmers field. Effective management of natural resources, integrated approach to plant-water, nutrient and pest management and extension of rapeseed-mustard cultivation to newer areas under different cropping systems will play a key role in further increasing and stabilizing the productivity and production of rapeseed-mustard. The paper reviews the advances in proper land and seedbed preparation, optimum seed and sowing, planting technique, crop geometry, plant canopy, appropriate cropping system, integrated nutrient management and so forth to meet the ever growing demand of oil in the country and to realize the goal of production of 24 million tonnes of oilseed by 2020 AD through these advanced management techniques.

1. Introduction

Rapeseed-mustard is the third important oilseed crop in the world after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq.) oil. Among the seven edible oilseed cultivated in India, rapeseed-mustard (*Brassica spp*.) contributes 28.6% in the total production of oilseeds. In India, it is the second most important edible oilseed after groundnut sharing 27.8% in the India's oilseed economy. The share of oilseeds is 14.1% out of the total cropped area in India, rapeseed-mustard accounts for 3% of it. The global production of rapeseed-mustard and its oil is around 38–42 and 12–14 mt, respectively. India contributes 28.3% and 19.8% in world acreage and production. India produces around 6.7 mt of rapeseed-mustard next to China (11-12 mt) and EU (10–13 mt) with significant contribution in world rapeseed-mustard industry. The rapeseed-mustard group broadly includes Indian mustard, yellow sarson, brown sarson, raya, and toria crops. Indian mustard (*Brassica juncea (L.)* Czernj. & Cosson) is predominantly cultivated in Rajasthan, UP, Haryana, Madhya Pradesh, and Gujarat. It is also grown under some nontraditional areas of South India including Karnataka, Tamil Nadu, and Andhra Pradesh. The crop can be raised well under both irrigated and rainfed conditions. Brown sarson (*B. rapa* ssp *sarson*) has 2 ecotypes lotni and toria. Yellow sarson (B. *rapa* var. *trilocularis*) is cultivated in Assam, Bihar, Orissa, and West Bengal as rabi crop. In Punjab, Haryana, UP, Himachal Pradesh, and Madhya Pradesh, it is grown mainly as a catch crop. Taramira (*Eruca sativa*) is grown in the drier parts of North-West India comprising the states of Rajasthan, Haryana, and UP. Gobhi sarson (*B. napus* L. ssp. *oleferia DC.* var *annua L.*) and karan rai (*Brassica carinata*) are the new emerging oilseed crops having limited area of cultivation. Gobhi sarson is a long duration crop confined to Haryana, Punjab, and Himachal Pradesh. It has good yield potential, wide adaptability and possesses high oil content of good quality. Karan rai yields well and shows better environment adoption and substantial resistance to pests and diseases. The country witnessed yellow revolution through a phenomenal

Table 1: Salient features of cultivated species of rapeseed-mustard (Cruciferous) group of crops.

SN	Common name	Botanical name	Days to maturity (days)	Yield potential, Kg/ha	Oil %
(1)	Indian mustard	*Brassica juncea*	105–160	1500–3000	38–42
(2)	Yellow mustard	*Brassica rapa* var. *yellow sarson*	120–155		41–47
(3)	Brown sarson	*Brassica campestris*	100–235	900–2000	40–45
		syn. B. rapa var. *brown sarson*			
(4)	Black mustard	*Brassica nigra*	70–90	1000–1200	40-41
(5)	Karan rai	*Brassica carinata*	150–200		36–43
(6)	Toria	*Brassica rapa* var. *toria*	70–100	600–1800	36–44
(7)	Taramira	*Eruca sativa*	140–150	700–1400	34–38
(8)	Gobhi sarson	*Brassica napus*	145–180	1300–2700	37–45

increase in production and productivity from 2.68 MT and 650 kg/ha in 1985-86 to 6.96 MT and 1022 kg/ha in 1996-1997, respectively. In spite of these achievements, there exists a gap between production potential and actual realization. In India rapeseed-mustard is grown on an area of 5.53 Mha with production and productivity of 6.41 MT and 1157 Kg/ha, respectively [1].

Mustard is cultivated in mostly under temperate climates. It is also grown in certain tropical and subtropical regions as a cold weather crop. Indian mustard is reported to tolerate annual precipitation of 500 to 4200 mm, annual temperature of 6 to 27°C, and pH of 4.3 to 8.3. Rapeseed-mustard follows C_3 pathway for carbon assimilation. Therefore, it has efficient photosynthetic response at 15–20°C temperature. At this temperature the plant achieve maximum CO_2 exchange range which declines thereafter. *Rai* is mostly grown as a rainfed crop, moderately tolerant to soil acidity, preferring a pH from 5.5 to 6.8, thrives in areas with hot days and cool night and can fairly sustain drought. Mustard requires well-drained sandy loam soil. Rapeseed-mustard has a low water requirement (240–400 mm) which fits well in the rainfed cropping systems. Nearly 20% area under these crops is rainfed. A review is prepared on advances on agronomic practices for enhancing the rapeseed-mustard production in India. A review of the work done on the different aspects in India and abroad especially under advance agronomic practices is done in this paper.

2. Crop Adaptation and Distribution

The rapeseed-mustard group includes *brown sarson, raya,* and *toria* crops. Indian mustard (*Brassica juncea* (L.) Czernj. & Cosson) is predominantly cultivated in Rajasthan, UP, Haryana, Madhya Pradesh, and Gujarat. It is also grown under some nontraditional areas of South India including Karnataka, Tamil Nadu, and Andhra Pradesh. The crop can be raised well under both irrigated and rainfed conditions. Being more responsive to fertilizers, it gives better return under irrigated condition. Brown sarson (*B. rapa* ssp. sarson) has 2 ecotypes *lotni* and *toria*. Yellow sarson (*B. rapa* var. *trilocularis*) is cultivated in Assam, Bihar, Orissa, and West Bengal as *rabi* crop. In Punjab, Haryana, UP, Himachal Pradesh, and Madhya Pradesh, it is grown mainly as a catch crop. Taramira (*Eruca sativa*) is grown in the drier parts of North-West India comprising the states of Rajasthan, Haryana and UP. *Gobhi sarson* (*B. napus l.* ssp. *oleferia DC*. Var. *annua* L.) and *karan rai* (*Brassica carinata*) are the new emerging oilseed crops having limited area of cultivation. *Gobhi sarson* is a long duration crop confined to Haryana, Punjab, and Himachal Pradesh. It is photo- and thermosensitive and makes little growth up to middle of February, but in the end of this month, plants make a quick growth. It has good yield potential, wide adaptability, and possesses high oil content of good quality. There are eight cultivated crops in rapeseed-mustard crop; the main characteristics features have been explained in Table 1.

Karan rai also yields well under a wide range of climate partly because it has a large number of primary and secondary racemes. It shows better environment adoption and substantial resistance to pests and diseases. Mustard is cultivated in most temperate climates. It is also grown in certain tropical and subtropical regions as a cold weather crop. Indian mustard is reported to tolerate annual precipitation of 500 to 4200 mm, annual temperature of 6 to 27°C, and pH of 4.3 to 8.3. Rai is mostly grown as a rainfed crop, moderately tolerant to soil acidity, preferring a pH from 5.5 to 6.8, thrives in areas with hot days and cool night, and fairly resistant to drought. Mustard requires good sandy loamy soil. The agro-climatic conditions of various locations under study have been explained in Table 2.

3. Varietals Development

Since, there is a vast variability in the climatic and edaphic conditions in the mustard growing areas of India, the selection of appropriate cultivars is important as it helps in increasing the productivity. Introduction of relatively short duration cultivar found favor with the environment where effective growing seasonal length is short. Improved varieties of mustard stabilize oil and seed yield through insulation of cultivars against major biotic and abiotic stresses enhance oil (low erucic acid) and seed meal (low glucosinolate) quality. The first Indian mustard hybrid, named "NRCHB-506," has been developed at Directorate of Rapeseed-Mustard Research, Bharatpur which can catapult the output of the country's key oil crop. The new hybrid is meant for cultivation in Rajasthan and Uttar Pradesh. Other high yielding varieties include "JM-1," "JM-3," and "Pusa Bold,"

Table 2: Agroclimatic conditions of various locations during mustard crop season.

Location	Longitude	Latitude	Temp, °C		Rain fall, mm	RH %		Soil texture	Soil fertility, Kg/ha		
			Max	Min		Max	Min		N	P	K
Hisar	75°43′6″ E	29°9′11″ N	3.2	34.2	50–200	38	96	Sany loam	130	12	480
Pantnagar	79°24′36″ E	28°58′12″ N,	4.8	32.3	150–400	47	92	Clay loam	155	15	310
Dholi	85° 35′22″ E	26°0′2.2″ N	6.6	33.3	200–550	52	94	Clay loam	140	12.5	275
Ludhiana	75°18′ E	30°34′ N	3.5	32.0	30–120	45	95	Loamy sand	150	24	220
Bhubneshwar	85°50′ E	20°16′ N	14.8	34.8	180–250	38	94	Clay loam	130	19	175

Table 3: Varieties tolerant to various abiotic and biotic stresses of mustard (*Brassica juncea*).

SN	Specific abiotic/biotic stress	Tolerant verities
(1)	Rainfed	Aravali, Geeta, GM 1, PBR 97, PusaBahar, Pusa Bold, RH 781, RH 819, RGN 48, Shivani, TM 2, TM 4, Vaibhav, RB 50
(2)	Salinity tolerant	CS 52, CS 54, Narendra Rai (NDR8501)
(3)	Frost tolerant	RGN 13, RH 819, Swaranjyoti, RH 781, RGN 48
(4)	High temperature tolerant	Kanti, Pusa Agrani, RGN 13, Urvashi, NRCDR 02, Pusa mustard 25 (NPJ 112), Pusa mustard 27 (EJ 17)
(5)	White rust resistant	Basanti, JM 1, JM 2, NRCDR-2
(6)	*Alternaria* blight tolerant	Jawahar Mustard 3, Him Sarson 1 (ONK 1), Ashirwad (RK-01-03)

"NRCDR-2," "NRCDR 601." Their yield potentials vary from 16 to 25 q/ha. At IARI, an early-maturing and bold seeded mustard variety has been developed called "Mehak" (*B. juncea*). This improved variety is suitable for early sowing to replace *toria (B. rapa* var. *toria)* in Delhi and adjoining areas. *Gobhi sarson* has a good yield potential, wide adaptability and possesses high oil content of good quality. "Hyola" (PAC-401) is canola type hybrid rapeseed, developed in India by Advanta India Ltd, Holland-based multinational company. "Neelam" (HPN-3) and "Sheetal" (HPN-1) are the popular varieties of *gobhi sarson* [2]. Since inception of mustard research programme in India, number of tolerant varieties to various abiotic and biotic stresses of rapeseed-mustard has been developed (Table 3).

"Pusa Jaikisan" of *B. juncea* is the first variety though tissue culture. "TL-15," a toria variety has been recommended as summer crop for high altitude of Himachal Pradesh. In an attempt to incorporate resistance/tolerance to biotic and abiotic stresses in high yielding varieties, aphid tolerant strains like "RH-7846," "RH-7847," "RH-9020" and "RWAR-842," *Alternaria* blight moderately resistant variety "Saurabh"; white rust resistant variety, "Jawahar Mustard-1"; salt tolerant varieties "Narendra Rai" and "CS-52" frost tolerant "RH-781" and "RH-7361" varieties have been identified. "RH-781" is also drought tolerant and suitable for intercropping. For nontraditional areas, Indian mustard varieties "Rajat," "Pusa Jaikisan" and "Sej.2" have been recommended.

4. Land and Seedbed Preparation

A mustard seedbed should be firm, moist, and uniform which allows good seed-to-soil contact, even planting depth and quick moisture absorption leading to a uniform germination. Tillage affects both crop growth and grain yield. The various tillage systems are as follows: conventional tillage includes moldboard ploughing followed by disc harrowing; reduced tillage includes disc ploughing followed by disc harrowing and complete zero tillage in which crop is sown under uncultivated soil. Minimum tillage, with or without straw, enhances soil moisture conservation and moisture availability during crop growth. As a consequence, the root mass, yield components and seed yield increase [3]. Zero tillage is preferred in mustard as it conserves more moisture in the soil profile during early growth period. Subsequent release of conserved soil moisture regulates proper plant water status, soil temperature, lower soil mechanical resistance, leading to better root growth and higher grain yield of mustard [4]. Success with minimum or zero tillage requires even distribution of crop residues, as a well-designed crop rotation and evenly distributing residue will create a firm, moist and uniform seedbed.

Continuous zero tillage results in redistribution of extractable soil nutrients with greater concentration near the soil surface, compared with conventional tillage where mixing of soil, residues, fertilizers, and lime results in a relatively homogeneous soil to the depth of tillage [6]. With zero tillage having greater root density in the surface soil but lesser root density below a depth of 15 cm in the soil profile. Therefore, P and K uptake by crops grown under zero tillage is greater than those grown by conventional methods. But the plant growth and dry matter yields of mustard under zero tillage will be higher only if N fertilizers are applied in appropriate amount [7]. Under AICRP on RM at Dholi, Kanke, Bhubaneshwar, and Behrampur maximum seed yield of *toria* and mustard was obtained in line sowing under zero

Table 4: Seed yield (kg/ha) and oil content (%) of *toria* as influenced by different N levels in *utera* cropping system at Bhubaneshwar.

Cropping system	N levels (kg/ha)		
	0	40	80
Rice: yellow sarson (broadcast) in *utera* cropping (at dough stage of rice)	428 (33.3)	823 (40.3)	810 (37.6)
Rice: yellow sarson (broadcast) in *utera* cropping (sowing before harvest of rice)	530 (30.2)	729 (38.2)	642 (37.1)
Rice: yellow sarson (line sowing) under zero tillage in rice field	506 (34.4)	924 (41.5)	886 (39.6)
Rice: yellow sarson (line sowing) after land preparation in rice fields	388 (32.5)	846 (40.4)	820 (38.4)
Rice: yellow sarson (broadcast) after land preparation in rice fields	301 (28.2)	460 (37.6)	440 (35.5)

CD at 5% cropping system: 79 (0.7), N levels: 32 (0.4), Cropping system × N levels: 98 (1.0). Figures in the parenthesis denotes oil content (%).
Source: AICRP-RM, 2003 [5].

tillage practice which indicated that mustard can be grown well under zero tillage.

At Bhubaneshwar, line sowing of mustard under zero tillage after rice gave the maximum seed yield (933 kg/ha) and oil content (38.4%) (Table 4). The soil under zero tillage system contains higher amount of organic matter having more carbohydrate, amino acid and amino sugar that results in qualitative and quantitative improvement in soil and soil structure due to least soil disturbance. Energy output and input ratio are higher in zero tillage as compared to conventional tillage.

5. Seed and Sowing

Vigorous seedling growth, good root development, early stem elongation, rapid ground covering ability, and early flowering and radiation are important yield determining traits under low temperature and radiation regime. These traits can be successfully exploited in mustard if a good seed is grown at appropriate time along with maintaining an optimum plant population.

5.1. Seed Priming. Seed treatment is a useful practice for healthy plant growth. Seed priming through controlled hydration and dehydration enhances early germination of mustard seed in less time, even in compacted soil [8]. The soaking of mustard seeds in 0.025% aqueous pyridoxine hydrochloride solution for 4 hours improved germination. The combination of pyridoxine + $N_{60}P_{20}$ + $N_{15}P_5$ (top dressing) accelerated the crop performance by enhancing seed yield and oil yield by 15.8 and 13.5%, respectively, over the control [9]. The differential response of varieties for imbibition gives advantage to some of them to germinate early as compared to others. At Hisar, maximum rate of imbibition was reported in "NRCDR-2" (41.7%) and minimum in "NRCDR-509" (7.5%). Such drastic difference in rate of imbibition is important for identification of suitable varieties under abiotic stress conditions namely drought, frost, and temperature abnormalities.

5.2. Sowing Time. Sowing time is the most vital nonmonetary input to achieve target yields in mustard. Production efficiency of different genotypes greatly differs under different planting dates. Soil temperature and moisture influence the sowing time of rapeseed-mustard in various zones of the country. Sowing time influences phenological development of crop plants through temperature and heat unit. Sowing at optimum time gives higher yields due to suitable environment that prevails at all the growth stages. Though different varieties have a differential response to date of sowing, mustard sown on 14 and 21 October took significantly more days to 50% flowering (55 and 57) and maturity (154 and 156) compared to October 7 planting [10]. Delayed sowing resulted in poor growth, low yield, and oil content. The reduction in yield was maximum in "RH-30" and minimum in "Rajat" [11, 12].

Date of sowing influence the incidence of insect-pest and disease also. Sowing on October 21 resulted in least *Sclerotinia* incidence [13]. The maximum (20.5–25.4°C) and minimum (3.9–10.7°C) temperatures at the flowering stage of crops established through sowing on October 21 were negatively correlated with the development of *Sclerotina* stem rot. Mustard aphid (*Lipaphis erysimi (Kaltenbach)*) has been reported as one of the most devastating pests in realizing the potential productivity of Indian mustard. Normal sowing (1st week of November) also helps in reducing the risk of mustard aphid incidence.

5.3. Planting Technique. Sowing technique depends upon land resources, soil condition, and level of management and thus broadcast, line sowing, ridge and furrow method and broad bed and furrow method are common sowing techniques. At higher soil moisture regimes, broadcasting followed by light planking gives early emergence and growth. Under normal and conserved moisture regime, seed placement in moist horizon under line sowing becomes beneficial.

At Shillongani, broadcast method was found to be more successful. Significantly higher seed yield of *toria (Brassica rapa var. toria)* was harvested in broadcast sowing of *toria* over other practices. *Toria* broadcast at dough stage along with 80 kg N/ha gave the highest yield (AICRP-RM, 2006). At Bhubaneshwar, line sowing of yellow sarson after land preparation produced maximum seed yield (870 kg/ha) with 40 kg N/ha [14]. At Behrampore, 40% higher seed yield of *toria* was obtained when sown in line after land preparation in the rice-based cropping system over broadcast (AICRP on RM, 2006). *Paira* or *utera* is a method of *cropping* in which the sowing of next crop is done in the standing previous crop without any tillage operation. Mustard sowing under *paira*/*utera* in the rice field has shown its edge over

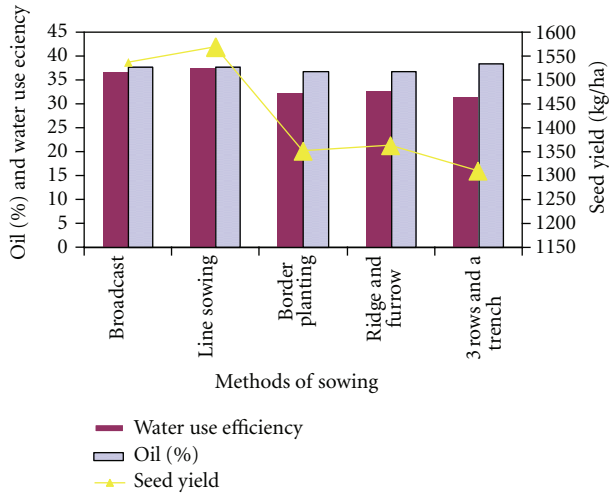

Figure 1: Seed yield, water use efficiency, Kg/ha-mm (WUE), and oil content of mustard (*Brassica juncea*) as influenced by various planting methods.

line sowing and broadcasting (Sowing of seeds by broad casting the seeds in the field) in eastern parts of India. At Dholi, mustard sown with *paira* cropping recorded significantly higher seed yield (1212 kg/ha) over line sown and broadcast method, while these 2 methods yielded at par. At Bhubaneswar, significantly higher yield (887 kg/ha) of mustard was recorded when sown as *utera* crop over line and broadcast sown crop [15].

Ridge and furrow sowing was superior to conventional flat sowing for growth parameters and yield of *Brassica juncea* [16]. Under saline condition, seed yield of canola in ridge sowing was higher by 45, 31, and 28% than broadcast, drill and furrow sowing methods, respectively [17]. The highest yield was associated with less saline environment at the ridges which allowed the seed to germinate and increase the yield. Transplanting of mustard has also been reported thereby saving time, and resources. Transplanting reduces days to maturity and results in higher seed yield. Ridge transplanting reduced water applied by 30% for each furrow as compared to 45 cm row spacing in flat method without any loss in seed yield. The corresponding increase in water use efficiency (WUE) was 27%. In bed planting, there was a 35% saving in water resulting in 32% increase in WUE (Figure 1).

5.4. Crop Geometry. The competitive ability of a rapeseed-mustard plant depends greatly upon the density of plants per unit area and soil fertility status. The optimum plant population density/unit area varies with the environment, the genotype, the seeding time, and the season. Uniform distribution of crop plants over an area results in efficient use of nutrients, moisture, and suppression of weeds leading to high yield. In wider row spacing, solar radiation falling within the rows gets wasted particularly during the early stages of crop growth whereas in closer row spacing upper part of the crop canopy may be well above the light saturation capacity but the lower leaves remain starved of light and contribute negatively towards yield.

Gobhi sarson (*Brassica napus*) being more vigourous, the days to maturity, plant height, branches, pod, seed weight per plant, seed index, seed yield, and oil content were higher at 60 cm row spacing [18]. An increase in rows up to 30 cm correspondingly prolonged maturity days followed by optimum 45 cm and wider rows 60 cm spacing. The plants receiving narrow row spacing increased vegetative growth. Due to shade and competition for nutrients and moisture the crop matures later by increasing developmental phases Taller plants were observed in the plots where crop was planted in rows of 60 cm apart followed by 45 cm and 30 cm row spacing due to sufficient space resulting in plants grown well and showed greater height [19] (Gupta, 1988). The regression coefficient indicated that each increase in row spacing up to 60 cm resulted in increased crop maturity by 0.54 days, plant height by 0.44 cm, number of branches would increase by 0.11, pods per plant by 1.96, seeds per pod by 0.04, seed weight per plant by 0.45, seed index by 0.152 g, oil content by 0.8% and increase in seed yield by 10.32 kg/ha. The recommended spacing for mustard is 30 × 10 and for hybrids it is 45 × 10. At Kumher, plant spacing 45 × 15 recorded significant higher seed yield over other spacing but was on a par with 45 × 10 cm. At Pantnagar, 30 × 15 recorded significantly higher seed yield which remained on a par with 45 × 10 and 45 × 15 cm plant spacing [20].

5.5. Plant Population and Inter-Plant Shading. The dense plant population reduces the yield due to reduction in the photosynthetically active leaf area caused by mutual shading. In an experiment on *Brassica juncea* (*Var. laxmi*) the reduction is more due to shading at 91–110 DAS over 71–90 DAS. The specific leaf weight (SLW), crop growth rate (CGR), and net assimilation rate (NAR) were more adversely affected by 50% shading at 71–90 DAS. Net assimilation ratio remained unaffected by 25% shading, while it reduced significantly by 50% shading at both the stages; the reduction was more with 50% due to shading at 91–110 DAS. On an average 50% shading at 91–110 DAS was more deleterious than 25% shading at 91–110 DAS, that is, at terminal seed development stage (Table 5).

6. Cropping System

Physiography, soils, geological formation, climate, cropping pattern, and development of irrigation and mineral resources greatly influence selection of variety and cropping system. Fallow mustard is popular sequence in major mustard growing areas but studies show that some of the crop result in better resource utilization and high remuneration if included in mustard-based cropping system.

6.1. Mustard Productivity under Various Crop Sequences. Under AICRP trials at Dholi, fallow-mustard sequence gave significantly higher seed yield which was on a par with blackgram-mustard sequence: urdbean-mustard at Morena; greengram-mustard, guar-mustard, and pearl-millet-mustard at S. K. Nagar and Hisar; maize-mustard

Table 5: Effect of shading on yield and growth parameters in Indian mustard at Hisar.

Treatment	Seed yield (kg/ha)	SLW (mg/cm^2)	CGR (g/m^2/day)	NAR (mg/m^2/day)
Control	571.6	8.3	11.3	0.93
25% shading at 71–90 DAS	546.0	7.0	10.8	0.93
25% shading at 91–110 DAS	490.9	7.9	9.4	0.87
50% shading at 71–90 DAS	527.0	6.4	9.9	0.95
50% shading at 91–110 DAS	380.0	7.0	8.3	0.83
CD at 5%	33.1	1.3	1.0	0.05

Source: AICRP-RM, 2004.

Table 6: Seed yield (kg/ha), mustard equivalent yield (MEY), and gross return (Rs./ha) as influenced by various intercropping combinations under rainfed conditions at Hisar.

Treatment	Main crop	Intercrop	MEY	Gross return (Rs./ha)
Pure mustard	2565	—	2565	29,497
Mustard + chickpea (1 : 5)	966	1035	1956	22,494
Mustard + fieldpea (1 : 5)	1002	189	1230	14,145
Mustard + linseed (1 : 5)	996	642	1721	19,791
Mustard + lentil (1 : 5)	1015	—	1015	11,672
Mustard without intercropping at same distance as in intercropping	1097	—	1092	12,668
CD at 5%	—	—	350	—

Source: AICRP-RM, 1997 [11].

at Kangra and Pantnagar revealed superiority to fallow-mustard. The productivity of the system also depends upon the fertility status and the nutrient supply. When mustard was grown after soybean or bajra, the response to S was observed up to 40 kg S/ha [21]. Productivity measured in terms of land equivalent ratio (LER) was higher for intercropping of chickpea and mustard in the 4 : 1 row ratio than for sowing of chickpea and mustard in sole stands [22].

6.2. Inclusion of Gobhi Sarson (Brassica Napus) under Various Cropping Sequences. *Gobhi sarson* is comparatively recent introduction and hence needs identification of suitable cropping systems. Growing *gobhi sarson* and toria in alternate rows at 22.5 cm spacing is very remunerative. Maize-*gobhi sarson,* blackgram-gobhi sarson, rice-*gobhi sarson,* and soybean-*gobhi sarson* were identified remunerative cropping systems at Kangra [21].

6.3. Mustard-(Brassica Juncea) Based Cropping System under Rainfed Areas. There are possibilities of increasing cropping intensity in monocropping mustard areas under rainfed condition. Green manuring or guar during rainy season enhance seed yield of succeeding mustard [12]. In addition to efficient resource use, intercropping imparts stability to productivity and reduces the risk of crop failure. Under irrigated conditions, at Bharatpur, the seed yield equivalent of mustard (*Brassica juncea*) was significantly higher where mustard was grown in combination with potato (1 : 3), mustard + wheat (1 : 5), mustard + barley (1 : 5) than pure mustard. At Hisar, intercropping *Brassica juncea* (variety RH-30) with rabi crops had revealed highest gross return (Rs. 29,498) when mustard was grown as a pure crop. The mustard seed equivalent was highest in mustard + chickpea (1 : 5). Intercropping of mustard with chickpea, field pea, or linseed proved superior over their cultivation as a pure crop (Table 6).

7. Fertilizer Management

Adequate nutrient supply increases the seed and oil yields by improving the setting pattern of siliquae on branches, number of siliquae/plant, and other yield attributes [23]. Recommended dose of fertilizers (RDF) for different zones changes with climate, soil type, time, and type of cropping system followed.

7.1. Nitrogen and Phosphorus Fertilization. Nitrogen use efficiency is greatly influenced by the rate, source, and method of fertilizer application. The rate of nitrogen depends upon the initial soil status, climate, topography, cropping system in practice, and crop. Crop under zero tillage is also more productive (695 kg/ha) with 80 kg N/ha [14]. Increase in the nitrogen level up to 60 kg N/ha consistently and significantly increased the number of primary branches, number of seeds per siliquae and 1000 seed weight [24]; however, increasing the nitrogen level up to 90 kg/ha increased the number of secondary branches per plant, number of siliquae per plant, and seed and straw yield with maximum cost benefit ratio of 3.03 [25]. Split application of total nitrogen in three equal doses one-each as basal, second after first irrigation and remaining one-third after second irrigation resulted in maximum increase in yield attributes and yield of *Brassica juncea* compared to application of total nitrogen in two split doses [26]. Top dressing of N fertilizers should be done immediately after first irrigation. Delaying of first irrigation, results in yield reduction of mustard crop. The application

Table 7: Effect of N and S levels (kg/ha) application on fatty acid composition and glucosinolate content in *Brassica juncea* cv. Varuna at Ludhiana.

N (Kg/ha)	S (Kg/ha)	Glucosinolate content (μmoles/g in defatted meal)	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
75	0	64	2.61	1.17	11.78	14.99	6.48	50.91	11.80
75	20	72	2.88	1.31	10.15	14.53	5.14	52.75	12.28
100	0	52	2.58	1.58	13.16	15.31	7.01	49.55	10.57
100	20	42	2.91	1.65	11.94	15.06	6.13	49.63	12.18
125	0	52	3.01	1.33	12.19	16.17	5.91	47.71	12.26
125	20	42	4.42	1.31	16.12	16.55	6.57	44.77	9.55

Source: AICRP-RM, 2007 [14].

of nitrogen with presowing irrigation was superior to that of nitrogen application with last preparatory tillage. In case of nitrogen applied with pre-sowing irrigation single application of nitrogen was on a par with split application [27].

Application of phosphorus up to 60 kg/ha significantly enhanced dry matter/plant. Plant height, branches per plant and leaf chlorophyll content increased with up to 40 kg P/ha. The uptake of NPK and sulphur by both seed and stover increased significantly with successive increase in nitrogen levels up to 120 kg N/ha, sulphur levels up to 60 kg S/ha, and P_2O_5 level up to 60 kg P_2O_5/ha. Seed yield and yield attributes increased while oil content decreased with increasing level of nitrogen up to 120 kg/ha. Different levels of phosphorus increased seed yield, maximum being at 80 kg P/ha due to higher number of secondary branches/plant and consequently siliquae/plant. Oil content also increased with increase in levels of N, P_2O_5, and S. Activities of all nitrogen assimilating enzymes, namely; nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate synthetase were found to be maximum at 100 kg N/ha.

7.2. Sulphur Fertilization. Among the oilseed crops, rapeseed-mustard has the highest requirement of sulphur [28]. Sulphur promotes oil synthesis. It is an important constituent of seed protein, amino acid, enzymes, glucosinolate and is needed for chlorophyll formation [29]. Sulphur increased the yield of mustard by 12 to 48% under irrigation, and by 17 to 124% under rainfed conditions [30]. In terms of agronomic efficiency, each kilogram of sulphur increases the yield of mustard by 7.7 kg [31].

Oil content in Canola-4 and Hyola-401 is 3% higher than the hybrid "PGSH-51" due to the effect of various doses of nitrogen and sulphur, while the oleic acid content in these hybrids is double that "PGSH-51." "PGSH-51" had erucic acid ranging from 23.2 in to 29.4%. At higher sulphur level there is 2-3% reduction in erucic acid content. However, lower level of nitrogen reduced erucic acid content by 3% with a concomitant increase in oleic acid (Table 7). Higher doses of sulphur along with low doses of nitrogen affect the chain elongation enzyme system thereby leading to reduction in erucic synthesis.

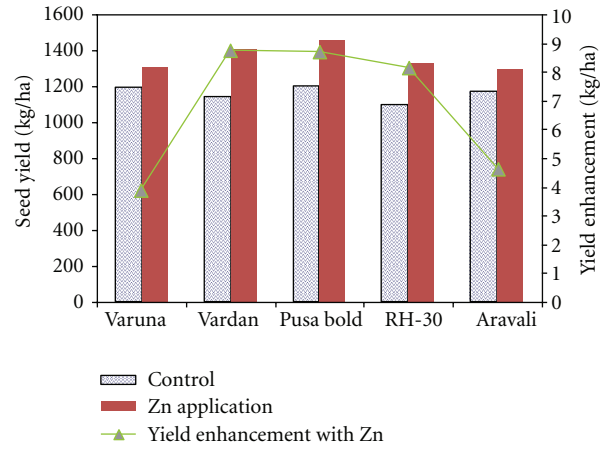

Figure 2: Influence of zinc application on seed yield of different cultivars of mustard.

A significant increase in yield was observed with increase in sulphur levels up to 40 kg S/ha in mustard-based cropping system. At Bawal, the highest seed yield of mustard was recorded in green gram-mustard cropping sequence while the lowest (2686 kg/ha) in pearl millet-mustard sequence. In rice-mustard sequence, the optimum seed yield of mustard was obtained at 40 kg S/ha at Behrampore and for blackgram-mustard at Dholi. Each successive increase in S level increased seed yield up to 20 kg S/ha at Dholi and Ludhiana, 40 kg S/ha at S. K. Nagar, and 60 kg S/ha at Behrampore and Morena conditions [32].

7.3. Micronutrients. Mustard, in general is very sensitive to micronutrient deficiency, specially zinc and boron. The increase in seed yield was 8.5% at 12.5 kg $ZnSO_4$/ha. The harvest index (HI) was significantly affected by Zn application, although seed yield showed diminishing return with additional $ZnSO_4$ doses (Table 8).

The response of various ideotype to the applied micronutrients varies considerably. The response of Indian mustard varieties, *viz.* 'Pusa Bold' and 'Vardan' to applied zinc was found higher (AICRP-RM, 2000) as compared to Varuna, RH- 30 and Aravali (Figure 2).

The concentration of Zn at flowering, pod formation stage, concentration and uptake of Zn in straw and grain

Table 8: Effect of Zn on yield and yield attributes of indian mustard.

$ZnSO_4$ (Kg/ha) levels	Seed yield (kg/ha)	Secondary branches/plant	Oil content (%)	Oil yield (kg/ha)	Protein (%)	Protein yield (kg/ha)	Harvest index (%)
0	1161	6.5	40.2	465.6	22.1	255.2	21.6
12.5	1260	8.1	39.9	501.1	22.5	281.9	22.4
25.0	1336	9.6	39.9	532.4	22.6	301.6	22.9
50	1414	12.4	39.9	570.0	22.5	318.6	22.2
CD at 5%	33	0.7	NS	22.8	NS	18.8	0.8

Source: AICRP-RM, 2000 [33].

at maturity and uptake of Zn in grain and straw at maturity of Indian mustard increased significantly with increase in Zn levels [34]. Similarly, the seed yield increased significantly (16–47%) with the application of boron. The average response to boron application ranged from 21 to 31%. The yield increase was due to 27% and 10% increase, respectively, in seeds/siliqua and 1000 seed weight, indicating the importance role in seed formation [35, 36].

7.4. Organic Sources of Nutrients. Bulky organic manures are applied to improve overall soil health and reduce evaporation losses of soil moisture. Depending upon the availability of raw material and land use conditions various organic sources, *namely,* clusterbean (green manure), *Sesbania* (green manure), mustard straw @ 3 t/ha and Vermicompost (2.5–7.5 t/ha) have been evaluated at Bharatpur. Green manure with *Sesbania* gave significantly higher mustard seed yield at Bharatpur and Bawal. *Sesbania* green manuring has shown higher mustard yield and improved soil environment (AICRP-RM, 2006).

Many biostimulants also encourage higher production. At Hisar, foliar spray of Bioforce (an organic formulation) 2 mL/L at the flowering and siliqua formation stage enhanced mustard seed yield (2059 kg/ha) [14].

7.5. Integrated Nutrient Management (INM). It is important to exploit the potential of organic manures, composts, crop residues, agricultural wastes, biofertilizers and their synergistic effect with chemical fertilizers for increasing balanced nutrient supply and their use efficiency for increasing productivity, sustainability of agriculture, and improving soil health and environmental safety. Balanced fertilization at right time by proper method increases nutrient use efficiency in mustard. Experiments have been conducted at different AICRP centres with the integrated use of organic manure, green manure, crop residue, and biofertilizers along with inorganic fertilizers. INM not only reduces the demand of inorganic fertilizers but also increases the efficiency of applied nutrients due to their favourable effect on physical, chemical and biological properties of soil. The introduction of leguminous crops in the rotational and intercropping sequence and use of bacterial and algal cultures play an important role in increasing the nutrient use efficiency [37].

7.5.1. Growth Promoter, BioFertiliser as a Component of INM. Biofertilizers are inoculants or preparation containing micro-organims that apply nutrients especially N and P. Two types of N-fixing microorganisms namely free living (*Azotobacter*) and associative symbiosis (*Azospirillum*) and two P supplying microorganisms, namely, phosphate solubilizing bacteria and vesicular arbuscular mycorrhiza (VAM) were extensively tested at various AICRP-RM centers. Inoculation of mustard seeds with efficient strains of *Azotobacter* and *Azospirillum* enhanced the seed yield up to 389 and 305 kg respectively with 40 Kg N/ha. The total NPK uptake was also higher with *Azotobacter* inoculation. The combined application of 10 t FYM + 90 : 45 : 45 NPK kg/ha with *Azotobacter* inoculation gave the highest B : C ratio of 1.51. At lower N levels, without inoculation, the seed yield decline was more as compared to inoculated treatment. Growth promoter's formulations like bioforce and biopower contain bio-amino acid, plant growth promoting terpenoid, siderophores, and attenuated bacteria fortified with BGA helped to increase water and nutrient absorption from the soil. Similarly, bioforce contains natural free amino acid, phytohormones, macro- and microelements and plant growth promoting terpenoid activated the cell division and stimulates plant growth, development, and photosynthate translocation. RDF (80 : 40 : 0) along with 25 kg Biopower/ha + spray of Bioforce (1l in 500 litres of water) at 50% flowering and pod filling stage gave significant higher yield of mustard over other combinations [35, 36].

7.5.2. Effect of INM on Quality of Mustard Oil. At Kanpur, INM studies were evaluated in maize-mustard, bajra-mustard, and fallow mustard sequence. In maize-mustard sequence, 100/75% of RDF + 2 t FYM gave highest seed yield and quality of the oil (Table 9).

7.5.3. Integrated Nutrient Management (INM) and Nutrient Use Efficiency. INM improves the nutrient uptake by mustard and hence enhances the use efficiency of various nutrients from the soil. The incorporation of 25% nitrogen through FYM + 75% by chemical fertilizer + 100% sulphur significantly enhanced the uptake use efficiency and of nitrogen and sulphur in both seed and stover of crop followed by 100% NS and 50% N through FYM + 50% by chemical fertilizer + 100% S [38]. The highest mustard-equivalent yield, which includes converted yield of other crops in to mustard seed yield based on market price of the crops (24.88 q/ha), net monetary returns (Rs. 15,537/ha), B : C ratio (2.07), and agronomic efficiency (16.1) were

Table 9: Effect of INM on quality of mustard (Kanti-RK 9807) under maize-mustard sequence.

Treatment	Legends	Oil content (%)	Fatty Acid composition (%)					
			16 : 1	18 : 1	18 : 2	18 : 3	20 : 1	22 : 1
			Palmtic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
RDF (120-40-40)	T1	40.4	2.8	18.4	10.1	10.6	4.3	52.7
T_1 + 10 t FYM/ha	T2	40.9	2.8	16.3	13.3	10.4	4.1	52.2
T_2 + 40 Kg S/ha	T3	40.4	2.9	18.0	14.4	12.2	3.2	48.6
T_3 + Zn SO4 25 kg/ha	T4	40.3	2.8	17.8	14.9	10.1	6.1	47.3
T_4 + B 1 kg/ha	T5	40.7	2.7	23.0	16.2	9.0	5.2	43.3
T_1 + Crop residue (Maize)	T6	40.1	2.7	20.0	14.3	9.2	4.4	48.6
75% RDF		40.4	2.6	17.8	15.1	7.9	6.3	49.7

Source: Modified from AICRP-RM, 2002 [21].

recorded with the application of 100% recommended N in the rainy season through FYM and 100% recommended NP in the winter season through inorganic fertilizers [39]. Agronomic efficiency is the response in terms of increase in mustard seed yield per unit use of nitrogen.

At Bharatpur and Jobner, 17.8 and 8.6% increase in seed yield was recorded with 50% RDF + 50% N through FYM and vermin-compost. Sole organic treated plot recorded 29.9% lesser seed yield over RDF at Jobner [32]. Amount of available phosphorus increased over initial value when organic manures and crop residues were incorporated. Organic carbon status builds up in organic source incorporated plots. The application of 10 t FYM/ha in addition to recommended dose of fertilizer (RDF) improved soil physical condition by improving aggregation, increased saturated hydraulic conductivity, and reducing bulk density and penetration resistance of the surface soil [40].

8. Water Management

Rapeseed-mustard crop is sensitive to water shortage. A substantial rapeseed-mustard area in Rajasthan (82.3%), Gujrat (98%), Haryana (75.6%), and Punjab (92.4%) is covered under irrigation. A positive effect of irrigating rapeseed-mustard at critical stages is observed. Water use efficiency was highest when irrigation was applied at 0.8 IW : CPE ratio and increased with increasing N rate [41, 42]. Number of irrigations is important for working out the most efficient water use by mustard. For mustard, two irrigations, one at flowering stage and at siliqua formation stage increased seed yield by 28% over the rainfed plots [43]. Increase in the amount of water increased leaf water potential, stomatal conductance, light absorption, leaf area index, seed yield, and evapotranspiration and decreased canopy temperature [44]. In similar study by Panda et al. [45], an average increase in seed yield with irrigation at the flowering and pod development stages and irrigation at the flowering stage over the control was 62.9% and 41.7%, respectively. However, for number of seeds per siliqua and oil content, single irrigation at 45 DAS remained parallel with two irrigations [46]. The water use efficiency was highest with one irrigation at 45 DAS. Crop receiving two irrigations at preflowering and pod-filling stages produce about 33 percent more seed than unirrigated crops [47]. Single irrigation given at vegetative stage is found to be most critical, as irrigation at this stage produces the highest yield. When two irrigations are given, the irrigation at vegetative and pod formation stages is of maximum benefit. The irrigation at vegetative, flowering, and pod formation stages resulted in the highest yield, where three irrigations were given. Oil and protein yield were also significantly affected by number and stages of irrigation (Table 10).

Irrigation is very important for getting the optimum productivity potential of mustard, but equally important is the quality of irrigation water. If the quality of irrigation water is poor, it needs certain treatment and management before being utilized for crop production. The increasing levels of salinity of the irrigation water applied at presowing and flower initiation reduces the plant height, the branching pattern, and the pod formation [48]. Irrigation with saline water (12 and 16 dS/m) decreased the dry matter yield significantly when applied at pre-sowing or later. The saline irrigation at the pre-flowering stage or later reduced the grain yield by 50% and 70%, respectively.

As a result of saline water irrigation, the soil water infiltration was reduced up to 7%. The EC and exchangeable sodium percentage (ESP) were increased by 2.2 dSm^{-1} and 9.0, respectively. The yield of mustard crop could be further increased by better leveling the plots, reducing the level difference to less than 10 cm [49]. The ill effects of saline water can be overcome with proper N management. Nonsaline water can be substituted by applying N and saline water [50].

9. Weed Management

Weeds cause alarming decline in crop production ranging from 15–30% to a total failure in rapeseed-mustard yield. The critical period is 15–40 days. Weeds compete with crop plants for water, light, space, and nutrients. Therefore, timely and appropriate weed control greatly increases the crop yield and thus nutrient use efficiency. The common weeds of mustard are *Chenopodium album, C. murale, Cyperus rotundas, Cynodon dactylon, Melilotus alba, Asphodelus tenuifolius, Orobanche* spp. *and Anagallis arvensis.*

Farmers have adopted herbicides for weed control because the chemicals can increase the profit, weed control efficiency, production flexibility and reduce time and

Table 10: Influence of irrigation levels and stages on seed yield, oil yield and protein yield of Indian mustard.

Treatment	Seed yield (Kg/ha)	Oil yield (kg/ha)	Protein yield (kg/ha)
4 irrigations at V + F + P + S	2260	909	454
3 irrigations at V + F + P	2250	901	454
3 irrigations at V + F + S	2200	886	442
2 irrigations at V + P	2150	879	436
2 irrigations at V + F	2090	841	422
2 irrigations at F + P	2020	803	417
2 irrigations at P + S	1520	574	316
1 irrigation at V	1920	773	386
1 irrigation at F	1790	727	371
CD at 5%	480	144	94

Note: V: vegetative stage; F: flowering stage; P: pod formation; S: seed development.
Source: AICRP-RM, 1999 [15].

labour requirement for weed management. Hand weeding at 20DAS, fluchloralin preplant incorporation @ 0.75 kg/ha, wooden hand plough between the lines at 35 DAS on Indian mustard was found effective [51]. Polythene mulch was also found effective in controlling the weeds in mustard [52]. At Bawal, reductions in weed population and dry matter were obtained with fluchloralin supplemented with hand weeding at 30 and 60 DAS, which remained on a par with isoproturon and pendimethalin supplemented with hand weeding at 30 and 60 DAS. Weed-free plot recorded 39.9% higher seed yield over weedy check [32].

Broomrape (*Orobanche*) is a major devastating parasitic weed of mustard. Broomrape weed infestation caused 28.2% average reduction in Indian mustard yield. Among *Orobanche* spp., *O. aegyptiaca* is one of the most important parasitic weed causing severe yield and quality reducing factor in rapeseed-mustard. It is endemic in semiarid region and may reach epidemic proportions depending upon soil moisture and temperature. Preceding crop of cowpea, black gram, moth bean, sunn hemp, cluster bean, and sesame significantly reduced *Orobanche* menace in succeeding mustard crop while sorghum, pearl millet, chilies, and green gram did not influence broomrape infestation in mustard [53]. At Bharatpur, S. K. Nagar and Bawal directed spray of glyphoste (0.25–1.0%) and 2 drops of soybean oil per young shoot of *Orobanche* showed effective control and recorded 91.9% higher seed yield over infected sick plot.

Some cultural practices like mulching and hoeing are also helpful to curb some of the major weeds in mustard by providing a shield against sunlight, reducing the soil temperature and acting as a physical barrier for emergence of weeds. Maximum seed yield (2540 kg/ha) was obtained in the treatments where plots were kept weed-free followed by the treatment where mulching was done after hoeing (Table 11).

Table 11: Seed yield (kg/ha) and weed population/m^2 as influenced by different weed control practices.

Treatment	Seed yield	Weed population/m^2
Control	1620	57.0
Weed free (Khurpi)	2520	0.0
Hoeing at 25 DAS	2300	19.3
Mulching with bajra florets	1960	23.0
Fluchloralin @ 1 kg a.i./ha PPI	2000	23.0
Pendimethalin @ 1 kg a.i./ha PE	2050	22.1
Isoproturon @ 1 kg a.i./ha PE	1740	26.3
Hoeing at 25 DAS + mulching	2400	17.9
Fluchloralin @ 1 kg a.i./ha PPI + Hoeing	2210	20.3
Fluchloralin @ 1 kg a.i./ha PPI + Mulching	2100	22.5
Pendimethalin @ 1 kg a.i./ha PE + hoeing	2300	18.9
Pendimethalin @ 1 kg a.i./ha PE + mulching	1860	19.5
Isoproturon @ 1 kg a.i./ha PE + hoeing	1950	22.5
Isoproturon @ 1 kg a.i./ha PE + mulching	1910	22.9

Source: AICRP-RM, 2002 [21].

10. Response to Plant Growth Regulators

Plant growth regulators (PGR) involved in manipulating plant developments, enhancing yield and quality have been actualized in recent years. Indeterminate plant growth habit, shattering, or dehiscence of fruits and lodging are the most significant and consistent limitations to maximum seed yields in *Brassica* spp. Considerable seed loss takes place, before or during harvest, due to shattering of fruits, which is correlated with hormonal imbalances and poorly developed lignified cells in the fruit wall. Further, lodging of the crop canopy adversely affects seed quality and yield due to decreased photosynthesis, increased disease severity, impaired rate of drying, and reduced harvest efficiency. Chemical plant growth regulators are being increasingly used as an aid to yield enhancement [54].

Brassinolide is the most bioactive form of the growth-promoting plant steroid termed as Brassinosteroids. Biologically active brassinosteroids show high growth-promoting as well as antistress activity besides other multiple effects on

Table 12: Seed yield (kg/ha) and net returns (Rs./ha) of mustard as influenced by foliar application of agrochemicals at different locations.

Treatment	S. K. Nagar		Sriganganagar	Ludhiana		
	Seed yield (kg/ha)	Net returns over control	Seed yield (kg/ha)	Oil content (%)	Oil yield (kg/ha)	Glucosinolate (μ mole/g defatted meal)
Control	1707	—	1604	34.7	375	130
Thiourea (0.1%)	2087	3226	1696	35.9	429	142
S @ 40 kg/ha	2249	6712	1799	35.2	405	149
S @ 40 kg/ha + Thiourea (0.1%)	2039	4070	1883	33.4	411	134
Urea (2%)	2019	5409	1845	34.7	396	124
$ZnSO_4$ (0.5%)	1921	4622	1667	33.2	372	126
Boric acid (0.1%)	1928	3418	1650	34.3	387	115
CD at 5%	150	—	158	—	—	—

Source: AICRP-RM, 2003 [5].

Table 13: Effect of low monetary agrotechniques on seed yield and oil content of mustard at Bharatpur during 1997-1998.

Treatments	Seed yield (kg/ha)	% increase over local practice	Oil content (%)	Oil yield (kg/ha)
Local Practice (T1)	1200	—	40.3	463
RP (No thinning and gypsum) (T2)	1371	14.2	40.3	525
RP + thinning at 15 & 25 DAS (T3)	1407	17.3	40.5	560
T_3 + N-S sowing (T4)	1376	14.7	40.7	560
T_3 + Removal of 4th row and 4th plant (T5)	1156	3.7	40.4	467
T_5 + 56.75% N as top dressing (T6)	1073	10.6	40.3	432
T_3 + I irrigation at 40–50 DAS (T7)	1232	2.7	40.6	500
T_1 + 200 kg gypsum/ha (T8)	1217	1.4	40.9	500
T_3 + removal of 4 older leaves (T9)	1343	11.9	40.5	544
RP + de-topping at bud-initiation stage (T10)	1464	22	40.7	596

Source: AICRP-RM, 1998 [12].

growth and development. As botanical juvenile hormones, they enhance the growth of young plant tissue and stimulate in submicromolar concentrations metabolic, differentiation and growth processes. Brassinosteroid caused accumulation of maximum total dry matter as compared to rest of the treatment at physiological maturity.

NPK accumulation and yield were maximal when spraying of GA_3 was done at 40 DAS [55]. An increase in secondary and tertiary branching with consequent enhancement in seed yield through increased number of infloresence and siliquae per plant was observed with the application of Mixatalol (a mixture of long aliphatic alcohols varying in chain length from C_{24} to C_{32}) to *Brassica* plants as foliar spray [56]. The percentage of immature siliquae and shattering of siliquae decreased with this treatment. Mixtalol increased total dry matter of plants, partitioning coefficient, and harvest index. The contents of starch, protein, and oil were also higher in seeds from mixtalol treated plants.

The maximum plant height (169.1 cm), number of primary branches per plant (8.2), seed yield (2031 kg/ha), stover yield (5752 kg/ha), harvest index (26.1%), oil content (42%), and net returns (Rs. 20,471/ha) were recorded with thiourea (Shrama and Jain, 2003). At Bawal and Morena, highest seed yield (2060 kg/ha) was obtained with 40 kg S/ha + thiourea (0.1%). At Sriganganagar, significantly higher seed yield (1883 kg/ha) was recorded on a par with 40 kg S/ha + thiourea (0.05%), urea (2%), H_2SO_4 (0.1%), and 40 kg S/ha. 40 kg S/ha + thiourea (0.1%) resulted into 17.67% higher seed yield over no spray. The highest oil content (35.9%) was recorded with thiourea 0.1% spray. Glucosinolate content ranged from 115 to 154 (μmole/g defatted meal) in different treatment (Table 12).

11. Impact of Low Monetary Agrotechniques on Mustard Productivity

Agricultural inputs like fertilizer, irrigation, insecticides, pesticides, and herbicides, and so forth, are very expensive. Some nonmonetary or low monetary inputs can enhance the yield considerably with a slight increase in the cost of cultivation. There are a number of low monetary agro techniques which enhance the mustard yield considerably (Table 13). For harvesting the maximum yield of rapeseed-mustard at a given situation, all the production technologies, like, soil amendments, thinning, nutrient supply, sowing direction, irrigation, plant protection, and so forth should be planned well in advance. At Bharatpur, highest seed yield (1464 kg/ha) was recorded with the application of recommended practice (RP) + thinning at 15 and 25 DAS + detopping

at bud-initiation stage followed by RP + thinning at 15 and 25 DAS.

12. Future Line of Research

Rapeseed-mustard will continue to contribute considerably to the oilseed bowl of the country. A streamlined research programme for rapeseed-mustard should be focused on the below-mentioned points.

(i) Horizontal and vertical intensification in rapeseed-mustard production needs to be done for self-sufficiency in oilseed production. It is possible through varietal improvement and introduction of mustard in nontraditional areas.

(ii) An optimum agronomic package of practices for high yielding and insect, pest, and disease resistant varieties, along with the upcoming hybrids needs to be worked out.

(iii) Adoption of site-specific nutrient management (SSNM), precision agriculture, and conservation agriculture can bring more profits to the mustard growers.

(iv) An integrated weed management approach needs to be developed for problematic and parasitic weeds in mustard. *Orobanche* is becoming a serious constraint and for its management a holistic approach which includes GM techniques needs to be explored.

(v) Suitable crop models and simulation for various inputs like water and nutrients will be helpful to target the most productive and most potential mustard growing zones of India.

13. Conclusion

The tremendous increase in oilseed production is attributed to the development of high yielding varieties coupled with improved production technology, their widespread adoption and good support price. To meet the ever-growing demand of oil in the country, the gap is to be bridged through management techniques. The vertical growth in mustard production can be brought by exploiting the available genetic resources with breeding and biotechnological tools which will break the yield barriers. Horizontal growth in rapeseed-mustard can be brought in those rapeseed-mustard growing areas/districts of the country, wherever, the yield is lower than the national average. Production technologies for different agroecological cropping systems, crop growing situations like intercropping, salinity, rainfall, and so forth, under unutilized farm situations like rice-fallows, mustard to be followed after cotton, sugarcane, soyabean, and so forth, and mustard as a *paira* crop in rice with lathyrus, lentil or any other competing *rabi* crop in traditional and nontraditional areas, need to be worked out. It is estimated that at least 1 million hectares can be brought under cultivation, through adoption of such cropping systems.

Proper land preparation, proper time of sowing, selection of better quality seeds, and so forth are always neglected. Fertilizer application is little or nonexistent leading to poor productivity. Whether little is spent on fertilizer input goes entirely on nitrogenous fertilizers. This results in a big gap between requirement and production of mustard in India. Therefore site-specific nutrient management through soil-test recommendation based should be adopted to improve upon the existing yield levels obtained at farmers field. Optimum crop geometry, balanced NPK fertilizers, intercultural operations, and inclusion of farmyard manure are the building blocks for achieving the utmost yield targets of rapeseed-mustard. Effective management of natural resources, integrated approach to plant-water, nutrient and pest management and extension of rapeseed-mustard cultivation to newer areas under different cropping systems will play a key role in further increasing and stabilizing the productivity and production of rapeseed-mustard to realize 24 million tonnes of oilseed by 2020 AD.

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Research Article

Improvement of Soybean Oil Solvent Extraction through Enzymatic Pretreatment

F. V. Grasso,[1] P. A. Montoya,[1] C. C. Camusso,[1,2] and B. G. Maroto[1,2]

[1] *Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Avenue Velez Sarsfield 1200, 5000 Córdoba, Argentina*
[2] *Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Avenue Valparaíso s/no, 5000 Córdoba, Argentina*

Correspondence should be addressed to F. V. Grasso, fgrasso@agro.unc.edu.ar

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The purpose of this study is to evaluate multienzyme hydrolysis as a pretreatment option to improve soybean oil solvent extraction and its eventual adaptation to conventional processes. Enzymatic action causes the degradation of the cell structures that contain oil. Improvements in terms of extraction, yield, and extraction rate are expected to be achieved. Soybean flakes and collets were used as materials and hexane was used as a solvent. Temperature, pH, and incubation time were optimized and diffusion coefficients were estimated for each solid. Extractions were carried out in a column, oil content was determined according to time, and a mathematical model was developed to describe the system. The optimum conditions obtained were pH 5.4, 38°C, and 9.7 h, and pH 5.8, 44°C, and 5.8 h of treatment for flakes and collets, respectively. Hydrolyzed solids exhibited a higher yield. Diffusion coefficients were estimated between 10^{-11} and 10^{-10}. The highest diffusion coefficient was obtained for hydrolyzed collets. 0.73 g oil/mL and 0.7 g oil/mL were obtained at 240 s in a column for collets and flakes, respectively. Hydrolyzed solids exhibited a higher yield. The enzymatic incubation accelerates the extraction rate and allows for higher yield. The proposed model proved to be appropriate.

1. Introduction

Seed oils represent 70% of global oil production, of which 30% is soybean oil. Oilseeds are the most important export items in Argentina [1].

In oilseeds, the vacuoles within cells contain oil, and both cell walls and vacuoles have to be broken in order to improve solvent extraction. Therefore, the preparation of the seed before solvent extraction is critical to maximize oil recovery.

An alternative pretreatment to facilitate the release of oil from the seed could be enzymatic degradation. In this way, the partial hydrolysis of soybean seed cell structures with appropriate enzymes would increase permeability, which would in turn increase mass transfer [2]. An enzymatic treatment stage could be incorporated for industrial purposes without significant changes to conventional processes. The oil release obtained using this method could result in a higher extraction yield and/or smaller quantities of the organic solvents used [3].

In solvent extraction, pretreated oilseeds (porous solid matrix) come into contact with a pure solvent or a solvent mixture (miscella) to transfer the oil from the solid matrix to the liquid medium. While the principle of extraction is relatively simple, it is a complex mechanism [4]. In order to describe this process, the mass transfer phenomena involved and the eventual resistance to mass transfer in the solid phase (solid soybean) and in the liquid phase (hexane) should be analyzed. This process involves several phenomena: oil is diffused through the internal pores to the surface of the solid (internal mass transport) and is then passed to the bulk liquid by means of a convective mechanism produced by the concentration difference between the solution occluded in the pores and the bulk solution (external transport). Because the oil to be extracted is contained within an insoluble solid network with occluded miscella, the diffusion occurs mainly between the occluded solution and the solid, greatly affecting the extraction rate since the solid matrix resists diffusive transport [5].

The rate of conventive and diffusive mass transfer can be expressed by means of equations that predict that the transfer is proportional to the difference between the bulk and the liquid-solid interface concentration [6]. The mass transfer coefficient in the solid can be estimated from the effective diffusion coefficients, while the mass transfer coefficient in the liquid can be estimated from empirical correlations [7].

The purpose of this study is to evaluate multienzyme hydrolysis as a pretreatment option to improve soybean oil solvent extraction and, on the basis of the results obtained, to define a preparation stage adapted to existing industrial processes. For this evaluation, the optimum conditions for enzymatic treatment and the yield obtained in each case are determined first. Then, based on the reaction conditions optimized to maximize oil yield, the influence of the enzymatic hydrolysis on the diffusion as the rate-determining step is established. Finally, a mathematical model for column extraction was developed to describe the process as an approximation to the operation of an industrial system.

2. Materials and Methods

2.1. Materials. Soybean flake and collet samples obtained from the industrial plant of Aceitera Bunge Argentina S.A., located in Córdoba, Argentina, were used. Industrial-grade enzymes provided by Genencor International were used as follows: Spezyme FRED (α-amylase activity), OPTIDEX L-400 (glucoamylase activity), PEKTOZYME ULTRA C (pectinase activity), GC 440 (hemicellulase activity), MULTIFETC GC (cellulase activity), and MULTIFECT NEUTRAL (neutral protease activity). All enzymatic incubations were carried out in glass Erlenmeyer flasks in a rotary evaporator with temperature-regulated bath and agitation at 100 rpm. Analytical grade drugs supplied by Sigma and Merck were used. Buffer solutions pH 4.5, 5.0, 5.8, 6.6, and 7.2 were used. An analytical-grade n-hexane was used for extractions. The extractor used was a glass column with a reflux condenser to avoid solvent loss by evaporation and external jacket to control the temperature. The fixed bed was made up of flakes and collets with and without enzymatic hydrolysis. A peristaltic pump was used to supply the hexane.

2.2. Methods

Enzymatic Treatment. The solid sample was heated with buffer solution at working pH at 90°C for 15 minutes to inactivate the native lipase enzymes. The solid/liquid ratio was adjusted to 7 : 1. Each sample was treated with a multienzyme mixture (see Section 2.1) defined as optimal in previous studies [8]. Each sample was centrifuged at 3500 rpm for 5 minutes. The centrifuged suspension was divided into two phases: a liquid and a solid phase. Each hydrolyzed solid fraction was dried in an oven at 60°C to constant weight in order not to alter the solid sample and to reduce the moisture content, which should not exceed 11% in order to avoid negative effects on the extraction rate and efficiency [9].

Table 1: Natural variables and factor levels. Coded variables: X_1 = (T-50): 20; X_2 = (pH-5.8): 0.8; X_3 = (t-8): 4.

Natural variables	Factor levels				
	−1.682	−1	0	+1	+1.682
Temperature (T) (°C)	16.4	30	50	70	83.6
pH	4.45	5	5.8	6.6	7.15
Time (t) (h)	1.3	4	8	12	14.7

Optimization of Enzymatic Treatment. pH, temperature, and incubation time conditions for each assay were determined according to a central composite experimental design with 3 variables. The design consisted in 20 experimental runs for each solid. Table 1 shows the experimental conditions for each assay.

The experimental design was based on the Response Surface Methodology (RSM) and included replications of the central point to minimize variance of the regression coefficients and axial points (α 1.681), so as to obtain a rotatable design [10]. The least-squares method was used to estimate the parameters of polynomial approximation. The analysis of the response surface was carried out according to the surface set.

Effective Diffusion Assays. The assays to evaluate effective diffusion were performed using collets and flakes and hydrolyzed samples and following the procedure described above. A cloth cartridge containing between 2 and 3 g of each sample was placed into an Erlenmeyer flask. 500 mL of hexane was added and the liquid medium was stirred to eliminate external resistance to mass transfer. Each experiment was conducted at 25°C. At the end of time t, the cartridge with the sample was removed from the system. The experiments were carried out with 5-minute and 10-minute intervals until 1 hour of extraction was completed. Each sample was drained and dried to remove traces of solvent and moisture. After that, the samples were weighed and placed in a flask with pure hexane for batch extraction for 24 h. The cartridge and the sample were dried once again in an oven in order to weigh the oil-free sample. The difference in weight before and after batch extraction was taken as the oil content at removal time t out of the extractor.

Fixed Bed Column Extraction. The extractions to determine kinetics were carried out in a column, in which the fixed bed was made up of different soybean solids. The column was filled with these solids, which had been previously weighed. Pure hexane was used with temperature and flow defined from the top of the extractor. Miscella samples were taken at the exit of the extractor. The total extraction time was 60 minutes. 1 mL aliquots were taken at defined times. The oil in the miscellas obtained was determined spectrophotometrically at 280 and 300 nm, according to the required sensitivity [11].

Estimation of Effective Diffusion Coefficients. The system diffusion was determined according to Fick's second law, as

proposed by many authors [12–14], and Carrín and Crapiste [4]:

$$\frac{\partial c}{\partial t} = \mathrm{Def}\frac{\partial^2 c}{\partial x^2} \tag{1}$$

with the following initial and boundary conditions for an unlimited and perfectly agitated volume of bulk liquid:

$$\begin{aligned} &\text{for } t = 0, \quad c = c_o, \\ &\text{for } t > 0, \quad c = 0, \text{ in } x = -l,\ x = l. \end{aligned} \tag{2}$$

The solution to (1) was obtained for plate and sphere, according to the solid that was considered. The following was obtained by integrating each solution and taking into account that the series obtained converge rapidly.

For plate with thickness $2l$,

$$\frac{q}{q_0} = \frac{8}{\pi^2} e^{-\pi^2 (Dt/(2l)^2)}. \tag{3}$$

For sphere with radius r,

$$\frac{q}{q_0} = \frac{6}{\pi^2} e^{-\pi^2 (\mathrm{Def}\cdot t/r^2)}. \tag{4}$$

Because the triglycerides present in vegetable oil have different molecular weights and structures, it is easier to measure the amount of oil in relation to solid mass. Therefore, the c/c_o concentration ratio was turned into a q/q_0 quantity ratio in both equations [12]. Equations (3) and (4) were linearized and represented according to time t. The slope of both lines was used to evaluate the effective diffusion coefficients.

Mathematical Model for Column Extraction. The fixed bed was regarded as a section of an extraction column to which a steady stream of hexane, Q_{Lo}, is supplied and from which the same flow of miscella, Q_L, is extracted. The mass balances for each phase were the following.
Solid:

$$-\frac{dc_S}{dt} = \frac{k_S \cdot A}{V_S}(\hat{c_S} - \mathrm{Keq} \cdot c_L). \tag{5}$$

Liquid:

$$\frac{dc_L}{dt} = -\frac{Q_L \cdot c_L}{V_L} + \frac{k_L \cdot A}{V_L}\left(\frac{\hat{c_S}}{\mathrm{Keq}} - c_L\right). \tag{6}$$

In the mass balance estimation, it was assumed that the bed was made up of porous particles-isotropic and spherical particles for collets with and without enzymatic pretreatment and for flakes with enzymatic treatment. The oil content in each of the solids is uniform in all particles, and the oil behaves as a single component, since its triglycerides are highly soluble in hexane [12]. The solids contain macropores in which the oil globules reside; the solvent penetrates these pores and dissolves the oil instantly, forming the miscella (stagnant phase) [4, 15]. An equilibrium relationship is established between the oil content in the stagnant phase in the pores and the residual oil content in the solid. The oil transfer occurs from the pores to the miscella due to the oil concentration gradient. The column length-particle diameter ratio is high enough to neglect the radial concentration gradient. The porosities of the bed and particle are uniform and constant throughout the extraction process. No heat of mixing is produced, and the temperature is constant and uniform throughout the extraction. The equilibrium relationship determined experimentally includes the effect of solid moisture; the volumetric flow is constant because the flow of pure hexane supplied into the system is equal to the flow of miscella extracted from the system. The mass transfer constants in the liquid were estimated using the empirical correlation for fixed beds proposed by Geankoplis [6] and the mass transfer constants in the solid phase were estimated by equaling (5) (mass balance) with the equations that describe the diffusive phenomenon, taking into account the corrections due to material porosity [16]. The solution to (5) and (6) was numerically found using MatLab 2008a.

3. Results and Discussion

Optimization of Enzymatic Treatment. The amount of oil that can be extracted from soybean flakes using the Soxhlet method is 16% on the dry basis (DB), and 18.54% (DB) for soybean collets. An increase in yield is observed for all experimental runs with enzymatic pretreatment (see Table 2).

The RSM analysis enabled us to obtain the experimental conditions for the enzymatic aqueous pretreatment, through which the maximum theoretical yield in oil (% DB) is obtained for each type of starting material.

The ANOVA analysis (see Table 3) was used to define the polynomial coefficients of the response.

(a) Flakes. Both linear and quadratic effects of temperature were significant, exceeding 95% of confidence level in both cases, and so did the quadratic effect of incubation time. For the pH variable, the variation was not statistically significant ($P > 0.05$). It can also be inferred that there were no significant effects for interaction terms between variables. Therefore, the response function was defined as $Y(\%) = 27.055 - 0.06\,T - 3.68\,T^2 - 0.04\,t^2 + \text{error}$.

(b) Collets. The quadratic effects of temperature and incubation pH were significant, exceeding 98% of confidence level in both cases, and so did the crossover effect of pH and incubation time. For the other linear terms, the variation was not statistically significant ($P > 0.05$). It can also be inferred that there were no significant effects for interaction terms between the temperature and incubation time variables. Therefore, the response function was defined as $Y(\%) = 26.566 + 0.186\ \mathrm{pH}\cdot t - 3.10\,T^2 - 0.7274\,\mathrm{pH}^2 + \text{error}$.

As it can be observed, the P value indicates that the model is significant for all cases with more than 98% of confidence. On the other hand, the adequacy of the quadratic model with 98% confidence, a 1.347 total error for flakes and 0.864 for collets, and a nonsignificant lack of fit with a confidence level greater than 96% for the two starting materials was proved.

Table 2: Percent of oil yield for experimental design assays.

Assay	Coded variables			Yield Y (Oil %)	
	X_1	X_2	X_3	Flakes	Collets
1	−1.41	0	0	25.79	24.26
2	+1.41	0	0	21.39	21.58
3	0	−1.41	0	28.02	24.93
4	0	+1.41	0	25.53	25.30
5	0	0	−1.41	25.31	24.37
6	0	0	+1.41	26.85	26.01
7	−1	−1	−1	25.45	25.83
8	+1	−1	−1	22.00	25.46
9	−1	+1	−1	24.00	25.01
10	+1	+1	−1	23.62	23.19
11	−1	−1	+1	26.13	24.33
12	+1	−1	+1	21.55	23.11
13	−1	+1	+1	26.47	23.14
14	+1	+1	+1	23.87	25.98
15	0	0	0	27.88	26.19
16	0	0	0	26.28	26.73
17	0	0	0	26.34	26.78
18	0	0	0	26.47	26.38
19	0	0	0	28.46	26.65
20	0	0	0	26.62	26.68

For soybean flakes, the variable with the greatest influence is temperature; most enzymes used in the assays have similar optimum temperature ranges, which coincide with the maximum yield temperature. This yield is a direct function of the overall enzymatic activity, since it facilitates the release of oil through the degradation of cell structures. No significant variation in yield was observed in relation to the change in pH. This may be due to the narrow range of study and the great variety of enzymes used. Each of them has its own optimum pH: when one of them is at maximum activity, the others exhibit minor activity because they do not have their optimum pH. This assumption is strengthened by the fact that the extraction efficiency is not the result of the sum of individual activity [17]. For soybean collets, the three variables studied are significant to varying degrees. The greatest difference was observed for the pH variable in multienzyme hydrolysis. This may be due to the different structure of the solid. The soybean collets are obtained by combining hydrothermal and mechanical treatments. Heat-treated materials exhibit a greater influence regarding the pH variable, probably due to the fact that the effect of proteases on yield is more significant than carbohydrolase activity in this type of material [18]. The optimum pH for the proteases used is in the 5.5-6 range, while carbohydrolases exhibit optimum pH ranges closer to 5.

Table 4 summarizes the optimum conditions found for maximum oil yield for each of the solid materials tested. These optimal conditions were determined by deriving and setting the response function (stationary point) equal to zero.

Best yields were obtained for soybean flakes (27.59% DB); the combination of several pretreatments produces an improvement in yield. In the case of soybean collets, which go through solvent extraction directly, a lower yield (26.64% DB) was obtained when compared to flakes. These results agree with those obtained by Rosenthal et al. [18], who found higher extraction yields for non-heat-treated materials (flakes) than those for heat-treated materials (collets). This may be attributable to lower hydrolysis rates for heat-denatured substrates than those obtained with non-heat-treated materials [18].

Estimation of Effective Diffusion Coefficients. Table 5 shows the effective diffusion coefficients estimated for all materials without external resistance to mass transport and when the internal transport of material is due diffusional processes exclusively.

The coefficient values are calculated on the basis of the slope estimated for each of the fitted lines, linearizing (3) and (4). As it can be observed, the magnitude order of the estimated diffusion coefficients was between 10^{-11} and $10^{-10}\,m^2\cdot s^{-1}$, which coincides with previous data [19–27]. Varzakas et al. [5] compiled effective diffusion coefficients in magnitude orders between 10^{-12} and $10^{-10}\,m^2\cdot s^{-1}$ for a wide variety of solid foods, and so did Doulia et al. [19]. Diffusion through solids is more complex than diffusion through gases or liquids, that is why, in most cases, smaller diffusion coefficients are observed, and, therefore, mass transfer occurs more slowly. In all the cases studied, and considering unidirectional diffusion and plate and sphere geometries for the starting solid, oil extraction with hexane followed the model of nonstationary diffusion proposed by Fick's second law. It can be observed that the effective diffusion coefficient was higher for solids which have gone through more pretreatments, which is consistent with the degradation degree of cell structures. If the starting material is an enzymatically pretreated solid with degraded cell structures, the material has higher permeability to the extraction solvent and the movement of oil molecules into the bulk solution meets fewer physical barriers.

Mathematical Model for Column Extraction. The solution to (5) and (6) was obtained through the method of numerical differentiation formula with the MatLab 2008a tool. From these solutions, the simulated profile of oil content evolution in miscella at the column exit and the simulated profile of residual oil content evolution in the solid were estimated [7, 15, 16, 27, 28]. Figures 1 and 2 show the simulated data of oil concentration in miscella at the exit of the extraction column according to time for solid flakes and collets without enzymatic pretreatment, respectively. Figures 3 and 4 show the experimental and simulated profiles for solid flakes and collets with enzymatic pretreatment. Each Figure shows both the values obtained in experimental manner and those estimated with the model.

The figures show good correspondence between experimental data and values estimated with the model used for this experimental scheme and the extraction equipment

Table 3: ANOVA results for yield optimization in oil.

(a) Soybean flakes

	SS	FG	MS	F	P value
Model	67.089	9	7.454	5.534	0.0066
Error	13.471	10	1.347		
Total fit	80.569	19	4.240		
Quadratic fit	39.932	6	6.655	4.941	0.0134
Lack of fit	9.158	5	1.832	2.123	0.2401
Pure error	4.313	5	0.863		
Total error	13.471	10	1.347		

(b) Soybean collets

	SS	FG	MS	F	P value
Model	32.550	9	3.617	4.188	0.0178
Error	8.636	10	0.864		
Total fit	41.186	19	2.168		
Quadratic fit	30.609	6	5.102	5.907	0.0072
Lack of fit	8.364	5	1.673	30.729	0.0009
Pure error	0.272	5	0.054		
Total error	8.636	10	0.864		

Table 4: Optimum enzymatic incubation conditions to obtain maximum theoretical oil yield.

	pH	Temperature (°C)	Time (h)
Flakes	5.4	38	9.7
Collets	5.8	43.5	5.8

Table 5: Estimated effective diffusion coefficients at 25°C.

	Slope	Equivalent size (m)	Effective diffusion coefficient ($m^2 \cdot s^{-1}$)
Flakes (plate)	−0.0064	$l = 0.00045$	$0.88 \cdot 10^{-11}$
Collets (sphere)	−0.007	$R = 0.00375$	$1.67 \cdot 10^{-10}$
Hydrolyzed flakes (sphere)	−0.0084	$R = 0.00375$	$4.60 \cdot 10^{-11}$
Hydrolyzed collets (sphere)	−0.0111	$R = 0.00375$	$2.63 \cdot 10^{-10}$

R: sphere radius; l: plate thickness.

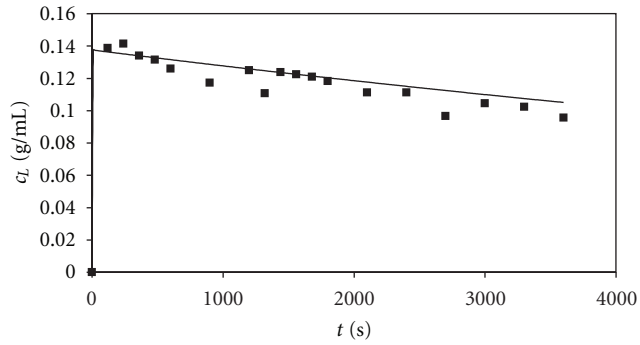

Figure 1: Evolution of oil content at the exit of the extraction column for flakes.

used. As it can be observed, the maximum amount of oil was obtained for the bed made up of hydrolyzed soybean collets. After 240 s of contact, the exiting miscella contained 0.73 g/mL of oil, compared to 0.7 g/mL of oil for hydrolyzed flakes. On the other hand, the graphs showed that the lowest value obtained corresponded to the bed made up of soybean flakes without hydrolysis. After about 2 minutes of extraction, the exiting miscella contained approximately 0.14 g oil/mL. It was observed that the maximum amount of oil obtained for soybean collets without hydrolysis (0.46 g/mL) is more than twice as much as the maximum value for soybean flakes without hydrolysis (0.14 g/mL). The difference could be due to the influence of the degree of cell structure destruction on oil diffusion in the solid, and it is a significant difference considering the high solubility of oil in hexane (solvent). Higher oil content was observed in the miscella at the exit of the column for shorter extraction times if hydrolyzed flakes are used instead of hydrolyzed collets. These results agree with the maximum yields obtained in the optimization stage.

4. Conclusions

The optimization of the pH, temperature, and enzymatic hydrolysis time variables led to maximum oil yield as follows: for flakes, incubation at pH 5.4 and 38°C for 9.7 h and, for collets, incubation at pH 5.8 and 43.5°C for 5.8 h. With enzymatic pretreatment, the oil yield obtained is greater than that obtained for conventional extraction without enzymatic

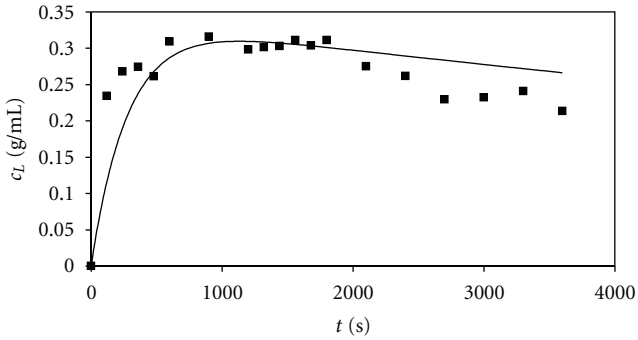

Figure 2: Evolution of oil content at the exit of the extraction column for collets.

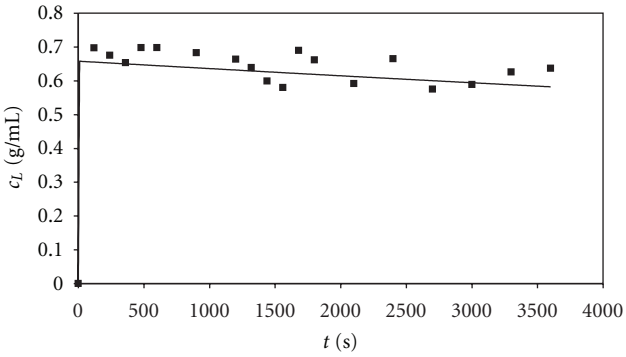

Figure 3: Evolution of oil content at the exit of the extraction column for hydrolyzed flakes.

treatment: 27.59% for soybean flakes, 26.64% for soybean collets.

It was determined that enzymatic hydrolysis decreases the integrity of plant tissues, increasing the permeability of these structures to oil, which results in greater diffusion coefficients for batch extraction in the absence of external resistance to oil transport.

The enzymatically treated soybean solids exhibited higher extraction rates and better yield for extracted oil. While the maximum rate of oil extraction with hexane in a fixed bed column is obtained when using enzymatically pretreated collets, the difference in extraction rate is not significant when compared to the use of hydrolyzed flakes.

The mass transfer phenomenon that determines the extraction rate was the oil diffusion that takes place within the solid. The proposed mathematical model of macroscopic balance proved to be appropriate to describe the fixed bed column extraction system.

Based on these conclusions, we can state that enzymatic hydrolysis is a new pretreatment option that could be incorporated into the current processes of soybean oil solvent extraction. The improvements obtained could be applied at industrial level and result in faster extraction processes, higher oil yield and/or decreased amount of solvent used.

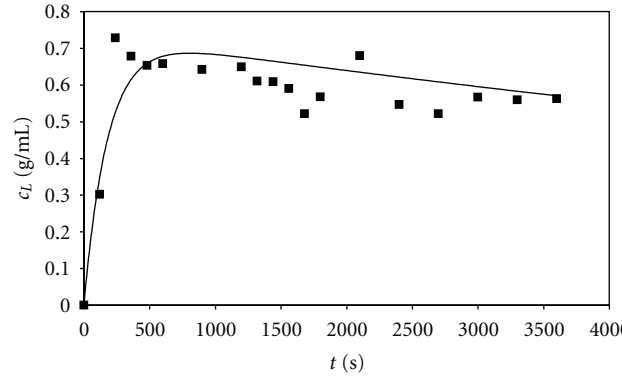

Figure 4: Evolution of oil content at the exit of the extraction column for hydrolyzed collets.

Nomenclature

A: Specific surface for mass transfer, $m^2 \cdot m^{-3}$
c: Oil concentration, $Kg \cdot m^{-3}$
D: Diffusion coefficient, $m^2 \cdot s^{-1}$
DF: Degree of freedom
F: Fisher test
k: Mass transfer coefficient, $m \cdot s^{-1}$
K: Equilibrium constant
l: Thickness of the plane sheet, m
MS: Mean squares
q: Oil mass, g
Q: Caudal, $m^3 \cdot s^{-1}$
r: Radius of the sphere, M
SS: Sum of squares
t: Time, s
V: Volume, m^3
x: Characteristic dimension for diffusion, m
Y: Oil yield.

Subscripts

ef: Effective
eq: Equilibrium
L: Liquid
o: Initial
S: Solid.

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Research Article

Effect of the Harvest Date on the Chemical Composition of Patauá (*Oenocarpus bataua* Mart.) Fruits from a Forest Reserve in the Brazilian Amazon

Raimundo Silva de Souza,[1] Jerusa Souza Andrade,[1,2] and Suely de Souza Costa[3]

[1] *Departamento de Tecnologia de Alimentos, Instituto Nacional de Pesquisas da Amazônia, 69060-001 Manaus, AM, Brazil*
[2] *Coordenação de Pesquisa, Universidade Nilton Lins, 69058-030 Manaus, AM, Brazil*
[3] *Departamento de Ciências Agrárias, Instituto Nacional de Pesquisas da Amazônia, caixa postal 478, 69060-001 Manaus, AM, Brazil*

Correspondence should be addressed to Jerusa Souza Andrade, andrade@inpa.gov.br

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This study aims to evaluate the effect of harvest date on the chemical composition of patauá (*Oenocarpus bataua* Mart.). Fruits were harvested monthly during the harvest season (June-December, 2009) from native plants in the Adolpho Ducke Forest Reserve located in Manaus, Amazonas, Brazil. The patauá was assessed for pulp yield and chemical composition. Variations in the bunch size, quantity of fruits, chemical constituents and calories occur throughout the season. The pulp yield showed two plateaus, the first from June to September and the second from October to December. The pulp yield was highest in the last three months, the amount of added water equilibrates the total solids and the lipids stood out as the major chemical constituent. At the end of harvest, the patauá became dry and oily and less fibrous. Despite the significant differences, considering that the pulp yield and solids content can be standardized by added water, the entire period of the season may be indicated for the patauá can be periodically collected and considered as a high-energy food for the people of Amazon.

1. Introduction

The Amazon has a great diversity of fruit species, and many of them are domesticated and present importance in the primary sector and commerce [1, 2]. Various palms native to Amazonia and other tropical regions of Latin America have been the subject of research and development and require sustainable extractivism [3]. This activity allows the exploitation of products from the forest and of biodiversity valorization [4, 5].

In the Amazon, the fruits of the palm trees such as açaí (*Euterpe oleracea* and *E. precatoria*), bacaba (*Oenocarpus bacaba*), buriti (*Mauritia flexuosa*), inajá (*Maximiliana maripa*), and patauá (*Oenocarpus bataua*) have similarities in the process of production, consumption, and consistency of pulps [2]. Before the pulping, all fruits should remain immersed in water slightly tepid to softening of the edible portion. Then, in pulping process is necessary the addition of the water. In the pulper occurs the scraping/pressing of the fruit (the edible portion is released and fragmented) followed by sieving (retention of the seeds and fragments non-crushed) and finally getting the pulp completely homogenized. This highly dense juice is popularly known as pulp or "wine" and consumed, added or not, of the manioc flour, "tapioca" flour, salt, or sugar [4, 5].

The other similarity between them is due to high content of unsaturated fatty acid in buriti, tucumã (*Astrocaryum vulgare*), inajá, mari (*Poraqueiba paraensis*), and patauá [6]. The color, nutritional value, and content of the bioactive substances vary due to differences in the chemical composition [7]: carotenoids in buriti [8]; anthocyanins in açaí (*E. oleracea*) [7, 9]; tocopherol in buriti and patauá [10].

Amazonian palm fruits are rich in fat. They have a fatty acid profile that may be beneficial in relation to risk of coronary heart disease. These fruits also contain other potentially cardioprotective constituents including tocopherol. Thus, they are good sources of unsaturated fatty acids and tocopherols [6]. Patauá pulp is highly oleaginous, and its fatty

acid composition is very similar to the ones of healthy oils, such as olive oil [10].

Among the Amazonian fruits we highlight the patauá. It is a fruit of a palm-stemmed. This palm tree has a height from 4.3 to 26 meters and a diameter of 15 to 45 cm. The fruits are ovoid, size from 2.5 to 3.5 cm, length from 2.0 to 2.5 cm in diameter, and color dark violet or purple [11, 12].

The patauá is principally used for pulp production. Besides the pulp, several researchers report that in the Amazon basis, traditionally the patauá oil is extracted and used for: preparation of food (fried foods, salad dressing, and meat preservative); cosmetic (prevent dandruff and hair loss); in traditional medicine (the oil is used to treat tuberculosis, ointment, decongestant, emollient, expectorant, and stimulant to bowel movements). The roots of the palm tree are used to combat diarrhea, worms, headache, and stomacal disorders [2, 12–14].

The patauá is harvested from native plants located in the forest according to the extractive system [15], and the pulp (principal use) and oil (minor use) are obtained from the fruit. The pulp is used as dense juice, and the oil is traditionally used in medicine, cosmetics, and foods. For oil extraction,the fruits are and heated with boiling water. The residue is discarded, and the liquid fraction (oil and water) is cooled. After decanting, the oil is separated from the aqueous fraction by density differences [13, 16].

The pulp is obtained from the pulper (powered by electricity), but the manual system is still used. The manual system consists of pressing the fruit in the container with water to release and grind the edible portion. Subsequently, the pulp is passed through a sieve. In the pulper, the process is discontinuous (batch). The fruits are smashed with water (added gradually). The pulp passes through sieve (disc kept inside in the pulper) and comes already mixed. The residue (seed and skin) is retained within the device and is subsequently removed. The amount of water is variable and depends on the traditional knowledge, the fruit maturation, and the yield desired.

Overall, the maturation of the fruit is important in terms of sensory, nutritional, and technological characteristics. The optimal harvesting point coincides with the maximum of chemicals constituents, which are responsible for these attributes. The dimension of the crop time allows the estimation of time required for dispersion of the species (dispersers natural) and for harvest. Thus, this estimate is the basis for the adoption of public policies to preserve the species and food availability.

In Brazil [17], Ecuador [15], and Colombia [18], the patauá phenophases were studied. With differences in periods and ecosystems, it was found that fruit production occurs throughout the year. However, besides the production of fruits is also important to evaluate the chemical composition of fruits throughout the season.

In Brazil, specifically in the Adolpho Ducke Forest Reserve, the same used in this study, was studied the phenology palm tree patauá, correlating phenological data (ten phenophases) with meteorological variables. Rainfall and sunshine were the variables with high discriminatory power in the formation of the main components. The bunches with ripe fruits occurred throughout the year, but in larger quantities from April to December [17].

In Colombia were studied the reproductive phenology and productivity patauá [18]. A phenological cycle the population level (since the appearance of flower buds to fallen fruit) takes approximately four years. Peak production of ripe fruits in a population occurred every 34 months. The behavior of the phenology of patauá is continuous and cyclical, with an approximate duration of four years. The low synchrony and the temporality of the production cycle indicated that there is no relationship between precipitation and phenology. The authors conclude that, to implement management programs and utilization of the species, it is important to consider that the production of mature fruits is not continuous and is presented for 22 months, alternating with 12-month supply void. Therefore, the sustainable management of forests through the periodic use can lead to food to local communities and even so, promote the conservation of ecosystems.

The importance of the studies on the patauá is to provide information on: biodiversity valorization; discloseure of the non-conventional oleaginous fruits; inclusion of this high-energy food in the specific diets; valorization of the family farms and the sustainable development of the Amazonian biodiversity; estimating the best harvest date. Thus, this study aims to evaluate the influence of the harvest date on the chemical composition of the patauá from the forest reserve located in the Amazon.

2. Material and Methods

2.1. Fruits Obtention. The fruits of patauá (*Oenocarpus bataua* Mart.) were harvested from native plants of the Adolpho Ducke Forest Reserve. This reserve belongs to the National Institute for Amazonian Research (Instituto Nacional de Pesquisas da Amazônia (INPA)) and it is located in Manaus, Amazonas, Brazil. It is situated along Highway 010 Manaus, Itacoatiara (km 26) and is bordered with the suburban areas of Manaus city. This reserve is situated between the coordinates 59°52′40″ and 59°58′00″, west longitude and 03°00′00″ and 03°08′00″, south latitude [19].

The reserve covers a continuous area with 10,072 hectares. The upland soils are interspersed with areas of lowland soils, and the natural vegetation is fully preserved. The patauá is found in the humid areas. The plants grow in high-density stands, some with about 100 plants [17, 19, 20]. The harvest was done in a population of 86 plants, located near one of the internal supervisory stations, called "Sabiá III."

Seven bunches (one bunch per month) of patauá (ripe fruits) were harvested from various plants during the harvest season (from June to December) of 2009 and the interval between each harvest was 30 days. Bunches were cut with a knife, carefully placed in the truck, and immediately transported to the Food Technology Department of the INPA, where this work was carried.

2.2. Obtention of the Pulp. The bunches, rachis fruits, and pulp were weighed, and the data were used to estimate the

Table 1: Physical characteristics of the patauá (*Oenocarpus bataua* Mart.) from the Adolpho Ducke Forest Reserve localized in Manaus, Amazonas, Brazil, and harvested at different times during the season (2009).

Harvest date	Physical characteristics						
	1	2	3	4	5	6	7
June	1.13	18.20	4.70	13.50	7.61	56.37	41.81
July	1.18	19.55	6.91	12.64	7.13	56.41	36.47
August	1.32	23.77	9.00	14.77	8.33	56.40	35.04
September	1.06	18.12	5.00	13.12	7.44	56.71	41.06
October	1.12	14.50	4.50	10.00	7.93	79.30	54.69
November	1.11	16.00	6.00	10.00	9.95	79.50	49.69
December	1.37	19.30	6.80	12.50	9.50	76.00	49.22
Average	1.17	18.49	6.13	12.36	8.27	65.18	44.00
SD	0.12	2.94	1.70	1.78	1.07	11.71	7.35
CV (%)	9.93	15.92	26.04	14.37	12.95	17.79	16.71

1: bunch lenght (m), 2: whole bunch weight (Kg), 3: rachis weight (Kg), 4: fruits weight (Kg), 5: pulp weight (Kg), 6: pulp yield (%) from the fruits, 7: pulp yield (%) from the bunche, SD: standard deviation, CV: coefficient of variation, means followed by the same letters within each column are not statistically different ($P < 0.05$, Tukey test).

pulp yield. The length of the bunch was measured with a tape measure.

In this study we used two methods of pulping: the system with mechanic pulper (electrically powered), thus obtaining the pulp P1; the manual system to obtaining the pulp P2. For both we used the same sequence of softening, moisturizing, pulping, and sieving. In this study the process (harvest and pulping) was aided by a person who knows and works with the extraction and sales of pulp of patauá.

The fruits were selected, weighed, washed, sanitized by immersion for 15 minutes in 0.02% sodium hypochlorite solution and rinsed with water. For the softening/moisturizing the fruits were separated into batches (±5 kg each), transferred to plastic containers (20 liters), and kept immersed for 30 minutes in water at 45°C. Then they were drained and pulped by two methods to get the pulp P1 (mechanical) and P2 (manual).

For the P1 (mechanical pulping) was used the pulper (stainless steel, electric-powered, capacity for 10 kg) with discontinuous system and each batch with ±5 kg. The process consisted of two sequential steps. During the first stage the equipment operated for three minutes with only the fruits. Then the operation continued for more seven minutes, but with the gradual addition of the water (distilled water). The total time for the process was 10 minutes (three minutes with only the fruits and then for more seven minutes after the water has been added). When out of the machine the pulp passed through the sieve (perforated disc at the bottom of the output of the pulper). The pulp was collected, homogenized, packed in plastic bags (500 g each), frozen, and stored in a freezer at −19°C.

In parallel, a part of the fruits was pulped using the manual method (still used in the Amazon region). The process consisted of the friction of the fruits in container (stainless steel pot), but without the addition of water. This pulp was also packaged in plastic bags. The pulp P2 (obtained with the manual system and no water added) was collected, homogenized, packed in plastic bags, and analyzed immediately.

2.3. Chemical Composition. The pulp P1 (obtained in the pulper and with addition of the water) was evaluated for moisture content and chemical composition. The pulp P2 (obtained with the manual system and no water added) was evaluated only on the moisture and dry matter. All determinations were performed in triplicate following the methods described by Ranganna [21]. The results are expressed as fresh and dry basis and reported as mean values ± standard deviation.

The moisture content was obtained by drying in an oven (with forced air) at 65°C until constant weight, the dry matter by difference and the ash by incineration in a muffle furnace for four hours at 550°C. The lipids were extracted with hexane for six hours in a Soxhlet apparatus. Total nitrogen was determined by micro-Kjeldahl method and converted to protein by use of the 6.25 factor. Fiber was determined using the Tecnal (TE-149 model) equipment. For acidic and alkaline hydrolysis, the 0.255 N H_2SO_4 and 0.313 N NaOH solutions were used.

The carbohydrates were estimated by difference. Gross energy (calories) was calculated using the AtVater method and the following values: protein = 4 kcal/g, lipids = 9 kcal/g, and carbohydrate = 4 kcal/g.

2.4. Statistical Analysis. The data were subjected to analysis of variance, and the Tukey test of means was applied with 5% of probability using ASISTAT software, version 7.2 [22].

3. Results and Discussion

3.1. Physical Characteristics. The results of the physical assessments are shown in Table 1. The bunch size (weight and length) and quantity of fruits showed variations throughout the season, and the highest values were detected in the third harvest (August).

The variations show the occurrence of diversity among the native plants, as previously reported [13, 15, 17]. The patauá showed large bunches, and the fruits are located in

Table 2: Moisture content of the patauá (*Oenocarpus bataua* Mart.) fruits harvested in the Adolpho Ducke Forest Reserve, in Manaus, Amazonas, Brazil.

Harvest date	Pulp obtention system	
	Manual	Mechanic
June	42.19 ± 0.28a	83.92 ± 0.12a
July	39.84 ± 0.26b	87.46 ± 0.33a
August	40.55 ± 0.57b	85.34 ± 0.71a
September	39.63 ± 0.35b	84.69 ± 0.28b
October	36.93 ± 0.42c	81.09 ± 0.40c
November	33.33 ± 0.72d	81.61 ± 0.17c
December	33.38 ± 0.33d	81.29 ± 0.59c
Average	38.06	83.54
SD	3.28	2.49
CV (%)	8.6	2.98

Means followed by the same letters within each column are not statistically different ($P < 0.05$, Tukey test).

Table 3: Chemical composition of the patauá (*Oenocarpus bataua* Mart.) fruits harvested in the Adolpho Ducke Forest Reserve, in Manaus, Amazonas, Brazil.

Harvest date	Chemical constituents (g 100 g^{-1} of the dry matter)			
	Lipids	Fiber	Protein	Ash
June	58.88 ± 0.68de	6.72 ± 0.19a	4.08 ± 0.04f	1.89 ± 0.11ab
July	57.26 ± 0.45e	6.69 ± 0.20a	4.87 ± 0.02e	1.61 ± 0.03b
August	58.90 ± 0.39d	5.48 ± 0.19b	5.92 ± 0.02c	1.89 ± 0.10ab
September	51.94 ± 0.57f	7.05 ± 0.45a	6.17 ± 0.13b	2.23 ± 0.18a
October	60.58 ± 0.40c	4.60 ± 0.18bc	6.79 ± 0.06a	1.63 ± 0.32b
November	70.21 ± 1.05a	3.87 ± 0.42c	5.31 ± 0.13d	1.74 ± 0.05ab
December	63.58 ± 0.11b	4.37 ± 0.29c	6.19 ± 0.12b	1.86 ± 0.32ab
Average	60.19	5.54	5.62	1.84
SD	5.39	1.26	0.88	0.26
CV (%)	9.0	22.81	15.70	14.1

SD: standard deviation, CV: coefficient of variation, means followed by the same letters within each column are not statistically different ($P < 0.05$, Tukey test).

the middle portion of the rachis. The pulp yield showed two plateaus, the first from June to September and the second from October to December. The pulp yield was highest in the last three months.

For pulping, the quantities of the fruit and the water were variable, according to the traditional knowledge. Even with difference in the characteristics of the fruit, they are able to obtain uniform pulp density. Despite variations (weights of fruits and pulp yield) during the season, the amount of added water equilibrates the total solids (dry matter). The amount of water to be added depends on the desired consistency, which was previously stipulated. Moving the pulp between the fingers (sensory) they evaluate and equalize the consistency of the pulp. In the Amazon, this is the normal process used by sellers of the patauá. Thus, the results in dry basis were used to evaluate the effect of harvest time.

3.2. Chemical Composition. The results of the moisture and the other chemical constituents are shown in Tables 2 and 3, respectively. The chemical constituents (expressed in dry matter) showed variations and with a significant difference between harvest dates. The moisture and fiber decreased during the season, while the protein and lipids increased. Minerals, represented by ash were relatively constant throughout the study period.

In addition to the higher content of proteins, at the end of harvest, the patauá became dry and oily and less fibrous. (Although not measured, knowledge of the depulper suggests the retention of the fibers in the machine sieve whose mesh is small, since it was designed for the pulping of açaí). Throughout the year, the region is characterized by two climatic periods: rainy (winter) and nonrainy season (summer) and beginning of fruit harvest (June) occurred after the end of the rainy season.

The moisture content (average = 38.06%) of the pulp (P2) from native plants of the Adolpho Ducke Forest Reserve is similar to patauá coming from the Colombia (36.9%) [23]. Natural pulp, obtained without water, is not obtained and consumed.

Despite the significant differences, considering that the pulp yield and total solids content can be standardized by added water, the entire period of the season may be indicated for the patauá can be collected.

Table 4: Nutritional value of the patauá (*Oenocarpus bataua* Mart.) fruits from the Adolpho Ducke Forest Reserve localized in Manaus, Amazonas, Brazil, and harvested at different times during the season.

Harvest date	Energy (Kcal) and nutritional constituents (g 100 g^{-1} of fresh pulp)			
	Lipids	Protein	Carbohydrates	Energy
June	9.47 ± 0.11d	0.66 ± 0.01e	4.57b	106.13c
July	6.92 ± 0.05g	0.61 ± 0.01f	3.97c	80.59e
August	8.63 ± 0.06e	0.87 ± 0.01d	4.08c	97.48c
September	7.95 ± 0.09f	0.94 ± 0.02c	5.00a	95.30d
October	11.46 ± 0.08c	1.28 ± 0.01a	4.99a	128.23b
November	12.90 ± 0.19a	0.98 ± 0.02c	3.48d	133.94a
December	11.89 ± 0.02b	1.16 ± 0.02b	4.49b	126.62b
Average	9.89	0.93	4.37	110.18
SD	2.12	0.23	0.52	20.59
CV (%)	21.43	25.09	11.84	

SD: standard deviation, CV: coefficient of variation, means followed by the same letters within each column are not statistically different ($P < 0.05$, Tukey test).

According to Miller [15], overexploitation of this species does not appear to be a problem. Nevertheless the potential for overharvesting exists and may also negatively affect frugivore populations, mammal and bird populations during food shortage periods, usually during transition from wet-to-dry seasons. Terrestrial rodent populations are especially important to dispersing and consuming the patauá.

As patauá is not alone and main meal, the disclosure of its importance in energy intake can increase the ecological awareness that it is a species that deserves to be properly exploited to improve the diet of human populations located in areas, where it occurs naturally.

3.3. Nutritional Value. The results of the nutritional value of patauá pulp are shown in Table 4. As the consumer purchases the pulp obtained in the puper (with water added) and standardized empirically, the result was also expressed as fresh pulp. Adding water (during the pulping process) increased the juiciness and decreased the amount of total solids. These solids (12.54–18.91%) are important because they contain chemical compounds with nutritional and functional value. The results of the nutritional components are higher than those found in açaí [24].

Results of the pulp P2 (without water) show that the patauá is not a juicy fruit. The mesocarp (pulp) and exocarp (skin) is oily and fibrous, respectively, and the patauá is not consumed as the flesh fruit. The obtention of the pulp from the patauá is similar to the others palm fruits such as açaí, buriti, inajá, and bacaba: it requires softening and moisturizing (immersion into the tepid water) in the first step and then, addition of water during the depulping. Thus, in the Brazilian legislation, the patauá is classified as tropical fruit, whose pulp can be incorporated with water.

The patauá is highly caloric, and the results are higher than buriti [25] and bocaiúva (*Acrocomia aculeata,* Jacq. Lodd. [26]). Throughout the season, there was an increase of lipids, and consequently, in the energy value. More than half (60.19%) of the chemical constituents are lipids, showing patauá as a rich source of energy belong to the so-called oleaginous crops. On balance the calories, the contributions of lipids, carbohydrates, and proteins were 80.6, 16% and 3.4%, respectively.

In all dates of harvest, the pulp of patauá (from the pulper, with water added) exhibited the following characteristics: dense consistency; high content of total solids; the appearance of an emulsion; purplish color and interspersed with small spots of the brown color; taste with equilibrium between sweet and acid, without the predominance of any of them.

4. Conclusion

Variations in the bunch size, quantity of fruits, chemical constituents, and calories occur throughout the season. The pulp yield showed two plateaus, the first from June to September and the second from October to December. The pulp yield was highest in the last three months, the amount of added water equilibrates the total solids, and the lipids stood out as the major chemical constituent. At the end of harvest, the patauá became dry and oily and less fibrous.

Despite the significant differences, considering that the pulp yield and total solids content can be standardized by added water, the entire period of the season may be indicated for the patauá can be collected and considered as a high-energy food for the people of Amazon.

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Research Article

Soybean Oil-Quality Variants Identified by Large-Scale Mutagenesis

Karen Hudson

USDA-ARS Crop Production and Pest Control Research Unit, 915 West State Street, West Lafayette, IN 47907, USA

Correspondence should be addressed to Karen Hudson, karen.hudson@ars.usda.gov

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To identify genetic variation for fatty acid composition in mature soybean seeds, 4566 M_3 generation seed samples from a chemically mutagenized population were subjected to fatty acid profiling by gas chromatography. In the population, a wide range of variation in the content for each of the five major fatty acids was observed. Seventy-nine lines were identified which contained significantly high or low levels of one of the five major soybean fatty acids. These lines were advanced to the subsequent generation. Of the 79 lines showing a variant fatty acid profile in the M_3, 52 showed clear heritability for the oil composition in the seeds of the subsequent generation. These lines are likely to represent 52 distinct genetic mutations. These mutants may represent new loci involved in the determination of soybean seed oil content or could be new isolates or alleles of previously identified genetic variants for soybean oil composition.

1. Introduction

Soybean oil is composed of five major fatty acids, which are synthesized in the seed during development [1]. In general, wild-type soybeans contain 10–12% palmitic acid (16 : 0), 3-4% stearic acid (18 : 0), 20–25% oleic acid (18 : 1), 50–55% linoleic acid (18 : 2), and 8–10% linolenic acid (18 : 3). Soybean oil has industrial uses and is also a source of vegetable oil for human consumption. For these divergent applications, a modified fatty acid composition profile is sometimes desirable. For example, oil low in the saturated fatty acid palmitate has health benefits in food oils, and reduction of linolenic acid results in increased oxidative stability without the need for hydrogenation [2]. However, for some industrial applications, increased levels of saturated fatty acids (stearate and palmitate) could be desirable [3]. Additional alleles providing new ways to incorporate new and existing oil composition traits could be of use to breeders for many reasons.

Genetic variation is an inexpensive and nontransgenic way to achieve alterations in oil content, which does not require postharvest processing. Soybean mutagenesis has previously been used for the successful improvement of seed oil composition [4–6]. In addition, novel alleles have been identified in germplasm collections of natural accessions, and the combination of these two sources of genetic variation has resulted in the identification of a number of soybean genotypes with modified oil content [2, 3]. In some cases genetic lesions underlying these traits have been identified at the molecular level. In all published instances for soybean, the mutations are found in genes encoding enzymes that function within the soybean oil biosynthetic pathway. A number of mutations in one of the major enzymes required for fatty acid biosynthesis in the developing soybean seed have been identified in current composition variants. Mutation in the gene encoding the beta-keto-acyl-ACP synthase (*KASIIa*) results in seeds containing high levels of palmitic acid [7]. The low linolenic acid-containing mutants of soybean carry mutations in the *fatty acid desaturase3* (*FAD3*) genes [8–10]. Lines carrying mutations in the omega-6 fatty acid desaturase (*FAD2-1a*) gene results in increased levels of oleic acid [11]. The A6 and FAM94-41 lines carry mutations in the gene encoding the delta-9-stearoyl-ACP-desaturase (*SACPD-C*), which results in high levels of stearic acid [12].

While many variants with significantly improved oil quality for various applications have been identified and used in breeding programs, previous efforts have focused on screening a population for a specific, favorable altered composition

trait. In this project we characterize and describe a large number of mutants with composition distinct from that of the parental genotype. In some cases variants with an unfavorable oil quality profile (one that is unsuitable for any current downstream applications) may add to the present body of knowledge about other genes required for normal oil deposition and soybean seed development and offer new avenues for the genetic improvement of oil content. At the time of this writing, all of the known mutants in oil composition traits have implicated biosynthetic enzymes, and it is likely that other genes encoding proteins that affect the activity or localization of these enzymes could impact oil quality. While such mutations may affect quality to a lesser degree, they may offer scope for more dramatic improvements using biotechnological approaches or in combination with other alleles. Here we describe the screening of a large mutant population for oil composition and the identification of a number of heritable mutations that affect the determination of soybean oil quality.

2. Methods

2.1. Plant Materials and Growth Conditions. The mutant population consists of N-nitroso-N-methylurea (NMU) mutagenized *Glycine max* cv. Williams-82 (W82) and was described previously [13, 14]. To screen for new, and as-yet unidentified, mutations that contribute to the fatty acid composition of mature soybean seeds, a forward genetic screen was conducted for seed oil composition. A total of 4566 mutagenized lines were subjected to fatty acid profiling by gas chromatography (GC). The fatty acid composition was initially screened by profiling samples of M_3 seeds (each sample consisting of three seeds from an individual M_2 plant) produced in the field at West Lafayette, IN, USA during the 2005 growing season and comparing these to the W82 wild type grown in parallel. The heritability of the identified variants was subsequently confirmed in the next generation (5 M_4 seeds from 3 to 25 individual M_3 plants) produced in the field in West Lafayette, IN during the 2008, 2009, or 2010 growing seasons. To estimate the level of environmentally induced variation and to calculate statistical significance for plant-to-plant differences in oil content, ten W82 plants were harvested individually from the field (2009 growing season) and their seed was analyzed for oil content. Level of significance was calculated by Welch's t-test comparing values from M_4 samples to these ten wild-type seed samples.

2.2. Fatty Acid Composition Profiling. Fatty acid profiling was performed at the National Center for Agricultural Utilization Research (Peoria, IL) using the following protocol: fatty acids were extracted into $CHCl_3$: hexane : MeOH (8 : 5 : 2) for 4 hours at room temperature from three beans cracked with a small hammer. A volume of 0.1 mL 0.35 M methoxide was added to the samples. Samples were analyzed on an Agilent 6890 GC with an Agilent J&W GC column (DB225, 30 m × 0.25 mm id × 0.25 μm film thickness). Data was normalized such that the palmitic, stearic, oleic, linoleic, and linolenic fractions total is 100, and content is expressed as a percentage. Data was analyzed and plots were generated in Microsoft Excel and R (http://cran.r-project.org/). Product names are necessary to report factually on available data. However, the USDA neither guarantees nor warrants the standard of the product and the use of the names implies no approval of the product to the exclusion of others that may also be suitable.

3. Results

Screens for seed fatty acid content were conducted over four years. For each fatty acid, a normal distribution was observed among the 4566 lines profiled. Each distribution showed a number of outliers that potentially represent mutants with extreme levels of one of the major fatty acids (Figure 1). In the population as a whole, a broad range of composition of the five major fatty acids was observed. Specifically, palmitate levels ranged from 5 to 16%, stearate levels ranged from 2% to 15.3%, oleate levels ranged from 14.6 to 46.1%, linoleate levels ranged from 34.6% to 62.6%, and linolenate levels ranged from 4% to 12.9% (Figure 1). For each fatty acid, upper and lower cut-offs were chosen based on the distribution: nine lines with seed palmitate levels above 14% or below 7% were selected for further characterization. Eight lines with stearate content less than 3.5% or greater than 8% were selected, 39 lines with oleate content less than 19% or greater than 30% were selected, 5 lines with less than 40% or more than 60% linoleate were selected, and 18 lines with less than 5% or more than 10% linolenate were selected for further characterization. Mutant M_3 seeds of these 79 selected lines were planted in the field during the 2008–2010 growing seasons, and M_4 seed samples derived from self-pollinated individual plants from these lines were profiled for oil composition. Data from the M_4 seed samples allows determination if the phenotype was transmitted to the following generation, and if the M_3 lines were heterozygous or homozygous. Figure 2 shows the reproducibility of fatty acid levels in the M_3 and corresponding M_4 progeny seed samples for 52 lines that were selected for propagation and crosses on the basis of reproducibility of the phenotype in the M_4 samples, taking into consideration that the mutation may not have been homozygous in the M_3 generation. In particular, in three lines (lines 8, 34, and 36) evidence of both aberrant and wild-type fatty acid levels in the M_4 progeny is apparent, which may indicate that the original M_3 isolate was heterozygous. While a range of variation in the levels of fatty acids are observed in the M_4 progeny of several of the mutant lines, in contrast in the W82 control plants, variation between individual plants for the level of each fatty acid was less than 0.5%, and minimal year to year variation was observed (Figure 2). For the majority of selected lines, fatty acid values from mutant M_4 differed significantly from the wild type ($P < 0.001$).

Complete fatty acid profiles for the M_3 lines that were selected for further study are listed in Table 1, along with statistical significance data for oil content differences in the M_4 seed samples (see Section 2). A total of three (lines 1–3) low-palmitic acid-containing mutants were identified, two of these contained normal levels of oleic acid. Six (lines 4–9) high-palmitic acid-containing mutants were identified (see Section 4). Four low-stearic acid-containing mutants

Table 1: Complete fatty acid profiles for selected M_3 mutants.

Key ID	16 : 0%	18 : 0%	18 : 1%	18 : 2%	18 : 3%	Significance	
W82	10.6	4.0	22.9	54.8	7.1	NA	Wild type
1	5.9	4.4	31.2	53.2	5.4	***	Low 16 : 0
2	5.1	3.5	24.5	59.8	7.2	***	
3	6.6	4.6	26.8	55.6	6.4	***	
4	15.1	4.0	26.0	48.7	6.3	***	High 16 : 0
5	15.0	5.8	20.5	50.8	7.9	***	
6	12.4	5.0	23.3	50.8	8.5	***	
7	15.1	5.7	20.7	49.8	8.6	***	
8	14.9	4.8	22.5	50.8	7.1	NS	
9	16.4	3.2	19.3	52.9	8.3	***	
10	9.6	3.1	21.1	58.2	8.0	***	Low 18 : 0
11	10.3	3.2	21.0	58.8	6.7	***	
12	9.7	3.2	24.7	55.0	7.5	***	
13	8.1	2.9	23.7	56.0	9.4	***	
14	10.9	10.1	19.2	51.7	8.1	*	High 18 : 0
15	10.1	11.5	31.8	41.6	5.0	**	
16	8.6	15.3	17.2	50.9	8.0	***	
17	10.2	7.1	25.0	51.0	6.7	***	
18	9.5	3.9	17.4	58.5	10.7	***	Low 18 : 1
19	10.6	3.6	17.0	59.0	9.8	***	
20	10.1	3.7	16.5	62.6	7.1	***	
21	8.1	4.2	17.3	61.1	9.3	***	
22	7.7	3.6	17.7	60.6	10.3	***	
23	8.6	4.1	17.6	59.0	10.8	***	
24	8.4	4.3	46.1	34.0	7.2	***	High 18 : 1
25	10.7	5.3	38.0	39.6	6.4	***	
26	10.1	5.0	42.4	37.2	5.5	*	
27	9.7	4.4	34.3	46.0	5.7	***	
28	10.8	4.0	33.4	45.7	6.2	***	
29	8.4	4.8	41.5	39.6	5.7	**	
30	9.2	4.8	36.3	43.7	6.0	***	
31	9.8	4.7	36.4	41.5	7.7	***	
32	8.9	4.5	43.6	35.8	7.1	***	
33	8.9	4.4	35.7	45.4	5.7	**	
34	9.3	5.0	44.5	36.7	4.5	NS	
35	12.6	4.7	31.5	45.1	6.2	***	
36	9.1	4.7	33.2	47.8	5.4	NS	
37	9.2	4.8	38.5	42.0	5.5	***	
38	9.3	5.8	33.4	44.7	6.8	***	
39	9.9	4.3	33.7	44.5	7.7	***	
40	6.3	3.5	19.9	60.9	9.4	***	High 18 : 2
41	7.6	3.7	20.3	60.1	8.2	**	
42	9.6	3.5	19.9	61.0	6.0	***	
43	10.3	3.7	26.1	55.9	4.1	***	Low 18 : 3
44	9.0	4.8	37.8	44.4	4.0	***	
45	6.9	6.6	21.9	53.5	11.1	**	High 18 : 3
46	6.8	4.6	20.6	55.1	12.9	***	
47	10.1	4.4	24.0	50.0	11.5	*	
48	9.3	4.8	20.3	54.9	10.7	***	
49	10.7	4.4	19.2	55.0	10.8	***	
50	8.6	3.1	20.9	56.9	10.5	***	
51	9.4	5.2	16.8	56.1	12.6	***	
52	9.2	5.8	19.6	54.8	10.5	***	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant, NA: not applicable for wild type samples.

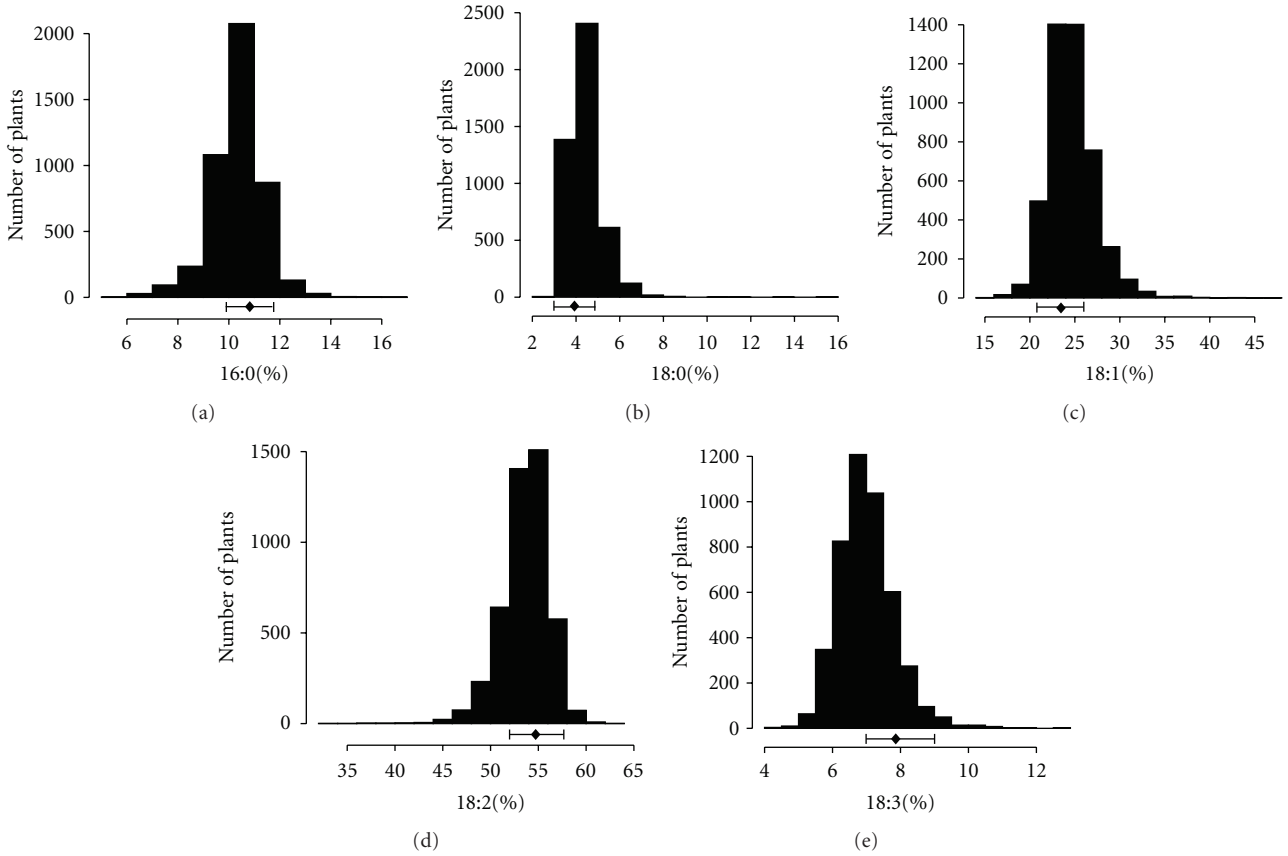

Figure 1: Distribution of fatty acid levels in the mutant population. Percentage of each fatty acid as measured over 4566 M_3 seed samples from mutant M_2 individuals by gas chromatography for five major fatty acids of soybean seeds. Black diamond on x-axis represents mean fatty acid level for Williams-82 wild type, and error bars represent two standard deviations from the mean. (a) 16 : 0 Palmitic acid, (b) 18 : 0 stearic acid, (c) 18 : 1 oleic Acid, (d) 18 : 2 linoleic acid, and (e) 18 : 3 linolenic acid.

(lines 10–13) and four mutants with high stearate levels (lines 14–17) were identified. Six low-oleate mutants (lines 18–23) had increased levels of linoleate, and five of these six had elevated levels of linolenate (in all cases these mutants would also have been identified as outliers in the population on the basis of linoleate or linolenate content). The high linoleate mutants (lines 40–42) had marginally reduced levels of oleate. All 16 high-oleate mutants (lines 24–39) had lower than average levels of linoleate. A decrease in linolenate occurred along with an increase in linoleate or oleate in two low-linolenate mutants (lines 43 and 44). All of the mutants with high levels of linolenate (lines 45–52) had reduced levels of oleate.

4. Discussion

Here we have described 52 newly isolated soybean seed composition mutants. These mutants may represent new alleles of previously identified loci that are known to be involved in the determination of seed composition, which will be determined by the sequencing of candidate genes encoding biosynthetic enzymes. Based on the mutagen used to generate these lines, the lesions in these mutants are predicted to be single-base changes. Alternatively, some of these mutants may carry lesions in genes not previously implicated in the determination of soybean seed oil content.

In this study, three mutants (lines 1–3 in Table 1) containing 5-6% palmitate were identified. Other mutants with low levels of palmitic acid (ranging from 5% to 8%) have been identified in previous studies, and at least three non-allelic loci are thought to be involved: *fap**, *fap1* (C1726), and *fap3* (A22) [15–19]. The allele fap_{nc}, which is allelic to *fap3*, has been associated with mutation of the *FAT1B* gene [19, 20]. Lines 4–9 contain high levels of palmitate, ranging from 12.4% to 16.4%. Mutants containing similarly elevated levels of palmitic acid have been described previously. The allele *fap2* has been associated with mutation of the *KASIIa* gene [7]. Other loci exist which confer a high palmitic acid phenotype which include *fap4*, *fap5*, *fap6*, and *fap7*. Therefore, there are likely to be other genes present that when mutated influence palmitic acid levels in the seed [16, 21–23].

Four mutants containing low levels of stearate (lines 10–13) were isolated in this study, however no mutants with this phenotype are characterized at the molecular level. Four mutants (lines 14–17) with reproducibly high levels of stearate ranging from 7 to 15% were identified in this study. Mutations in the *SACPD-C* gene are known to result in

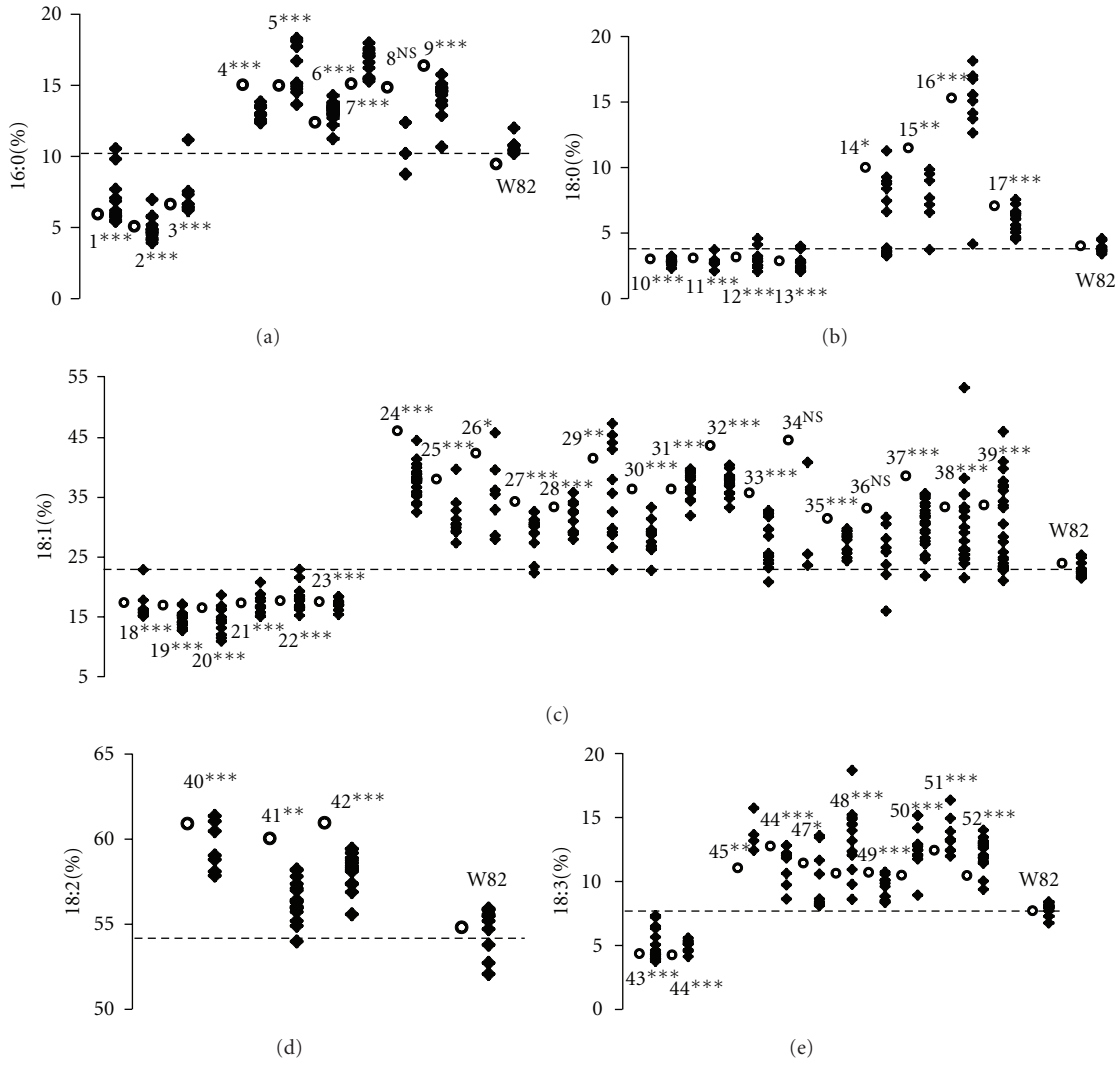

Figure 2: Reproducibility for fatty acid levels in selected mutant lines. For each M_3-M_4 pair the open circle on the left represents the fatty acid level observed in the M_3 seeds, and the black diamonds represent the levels observed in the M_4 seeds descended from M_3 individuals. Key for the labeling of each line is found in Table 1. The dotted horizontal bars on each plot represent the mean level of the fatty acid in wild-type Williams-82 (W82) seed samples. Actual values for a representative W82 sample (as well as values for single plant progeny from this W82 plant) is plotted on the far right of each plot for reference. Level of significance was calculated by Welch's t-test comparing values from M_4 samples to these ten wild type seed samples. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS: not significant. (a) Palmitic acid mutants (mean palmitic acid for W82 = 10.6%), (b) stearic acid mutants (mean stearic acid for W82 = 3.9%), (c) oleic acid mutants (mean oleic acid for W82 = 23.2%), (d) linoleic acid mutants (mean linoleic acid for W82 = 54.8%), and (e) linolenic acid mutants (mean linolenic acid for W82 = 7.7%).

increased levels of stearic acid ranging from 9% to 30% [5, 12, 24]. There are thought to be at least two genetic loci that contribute to the high stearate phenotype [25].

Sixteen mutants (lines 24–39) were identified in this study that contain high levels of oleic acid. Plants carrying deletions in the *FAD2-1a* gene contain high levels (up to 50%) of oleic acid in the seeds [11]. A number of other genes affecting oleate levels are known, but have not been characterized at the molecular level at the time of writing. One possible reason for this is that the dependence of oleic acid levels in soybean seeds upon environmental conditions complicates the molecular mapping of these genes [26–28]. RNA interference of *FAD2-1* (which probably silences multiple *FAD* transcripts in the seed) results in even higher oleate levels in the range of 70–80%, therefore it is thought that other *FAD* genes may contribute to the conversion of 18 : 1 to 18 : 2 [29, 30]. Six mutants containing low levels of oleic acid (lines 18–23) were also identified in this study.

Two mutants containing low levels of linolenate (line 43 and 44) were identified in this screen. Mutants with low linolenate (designated *fan*) have been identified previously, and two independent low linolenic mutants have been shown to contain lesions in the *FAD3A* gene [5, 10, 31, 32]. When all three *FAD3* homologs are downregulated with RNAi in seeds,

linolenic acid levels can be further reduced, therefore, it is possible that mutation in *FAD3B* or *FAD3C* may also result in low levels of linolenic acid [33]. Eight lines containing 10.5–12.9% linolenic acid were isolated in this study (lines 45–52). One mutant with comparable levels of linolenic acid (12.6%) is known, but the nature of the molecular lesion that causes this phenotype has not been determined [34].

5. Conclusions

Candidate gene sequencing and complementation tests or genetic mapping will be necessary to determine if the lines identified in this study represent new alleles of the known enzymes that control seed oil composition or represent mutations in other genes not yet implicated in the determination of soybean oil quality. These mutations have been isolated from a heavily mutagenized population created for the reverse genetics TILLING approach and carry a number of base changes at additional sites [13]. As with all variants created using a mutation breeding strategy, it will therefore be necessary to introgress these loci into elite cultivars for several generations to eliminate potentially deleterious mutations at other loci. Lines developed in this way will likely be of substantial utility for further development of new soybean varieties with both improved oil content and competitive agronomic characteristics.

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