Fatty Acid Composition of Sunflower in 31 Inbreed and 28 Hybrid

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ABSTRACT

High variability observed represents a very promising base to obtain new sunflower inbreds with high oil quality for use either as component in hybrids or as breeding stock for future generations. The high variability observed also suggests that selection in sunflower germplasm enable the creation of more oil profiles with different fatty acids. Further research has advanced to clarify the inheritance of fatty acids to generate superior hybrid. The important high accumulation of unsaturated fatty acids genotype are GP-01004, Entry no-20, GP-01009 and GP-04026 can be utilized further to develop a sunflower hybrids with high and good quality fatty acid. Among derived crosses, four crosses were found to be higher oleic acid content viz., GP-01009 x Entry P-S-2 (58.57%), BHAC-04038 (1) x GP-01005 (56.85%), Entry no.-20 x GP-01009 (53.36%) and BHAC-04038 (1) x GP-01009 (52.05%) compared to their respective parent (Table 1). Whereas involving parents GP-01009, Entry P-S-2, BHAC-04038 (1), GP-01005 and Entry no.-20 of these crosses contain 25.15%, 38.90%, 27.67%, 18.11% and 18.71% oleic acid (Tables 2 & 3) respectively can be utilized high oleic containing hybrid.

Introduction

Vegetable oil consists of different types of saturated and unsaturated fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid etc.). The palmatic acid and stearic acid are the major saturated fatty acids whereas oleic and linoleic acids are unsaturated. The sunflower oil contains more of unsaturated fatty acids, mainly oleic acid and linoleic acid and very less quantity of saturated fatty acid, palmitic acid and stearic acid. Healthy oil should contain more of unsaturated fatty acids compared to saturated fatty acids. High oleic oil is generally associated with the relative concentration of oleic and linoleic acid. Oil with high oleic acid content has several benefits with regard to human health. High levels of saturated fat (Palmitic and stearic acid) consumption are correlated with increased risk of coronary heart disease. Oils with high oleic content are resistant to heat oxidation, longer shelf life and low cholesterol effect. However, Roberston reported that linoleic acid content varied inversely with the oleic acid content.

High proportion of the essential fatty acid (18:2) is considered to reduce blood cholesterol and hence sunflower has a special significance. High linoleic sunflower with 70% linoleic acid is also available in studied germplasm. It has greater oxidative stability and is useful as frying oil in the preparation of snack foods. Furthermore, sunflower oil contains fat soluble vitamins A, B, E and K, well for heart proteins [1]. The objective of this study was to make a review of the genetic variability of oil quality components in sunflower and inheritance pattern in cross combination using own results and those of other authors.

Materials and Methods

Estimation of Fatty Acid Composition

Fatty acid profile of 31 inbreds and 28 F1 derived from selected eight parent through half diallel fashion was estimated by using gas chromatography. About 8-12 seeds was taken (seed was crushed in an oil paper and then transferred into a test tube). The sample was extracted and trans esterified at the same time with 5 ml ethylated reagent (petroleum ether/0.02M sodium hydroxide in ethanol (2/3) and shake. The samples were kept for overnight at room temperature. 10 ml salt solution (80 g NaCl and 3 g sodium hydrogen...
sulphate in 1 liter water) was added and shake. As soon as the two layers were separated, the benzene phase was transferred to small test tubes. A Philips PU 4500 chromatograph instrument was used with Flame Ionization Detector (FID). A glass column (1.5m x 4mm) was packed with BDS. With this column the injection post, column and detector temperature was set at 220°C, 185oC and 240°C, respectively. Nitrogen flow (used as carrier gas) rate was 22 ml/min, the injection volume was 2 μl. Peak areas were measured with an electronic digital integrator (Shinadzu C-R6A chromatopac).

Results and Discussion

The sunflower fatty acid profile includes estimation of various proportion of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), linolenic acid (C18:3) and cis-ecosenoic (20:1) expressed in terms of percentages. The results obtained for seven fatty acids of 31 inbreds line through gas chromatography are presented in Table 4. Total fatty acid composition (Saturated and un-saturated) of the different inbred lines presented in Table 2, fatty acid composition of selected inbred parents used in half diallel crosses in sunflower are presented in Table 1 and fatty acid profile of 28 F1 derived from selected eight parents are presented in Table 4.

**Table 1:** Fatty acid composition of the 31 inbred lines of sunflowers.

| Sl no. | Designation     | Palmitic Acid (C16:0) | Stearic Acid (C18:0) | Oleic Acid (C18:1) | Linoleic Acid (C18:2) | Linolenic Acid (C18:3) | Cis-ecosenoic (20:1) |
|--------|-----------------|-----------------------|----------------------|---------------------|-----------------------|------------------------|---------------------|
| G1     | BHAC-SH-037     | 6.2311                | 1.9822               | 0.2011              | 34.5679               | 55.6783                | 0.0459              |
| G2     | Sun-W-101       | 7.7211                | 5.4484               | 0.4336              | 31.1099               | 54.0554                | 0.1173              |
| G3     | Sun-W-103       | 6.0801                | 2.0269               | 0.2584              | 44.7745               | 45.2137                | 0.0852              |
| G4     | BHAC-O4032      | 7.9893                | 1.4061               | 0.2909              | 22.2583               | 67.3469                | 0.1323              |
| G5     | BHAC-P-5-2      | 8.4618                | 1.9625               | 0.4207              | 25.5723               | 62.5897                | 0.0909              |
| G6     | BHAC-O4038(1)   | 6.0567                | 1.7843               | 0.4563              | 27.6745               | 63.8763                | 0.0875              |
| G7     | BHAC-O4026      | 8.5301                | 0.8514               | 0.3846              | 28.3661               | 60.7532                | 0.1201              |
| G8     | BHAC-O4028      | 6.0432                | 1.4194               | 0.4843              | 40.3115               | 50.5877                | 0.0594              |
| G9     | BHAC-O4016      | 6.9573                | 3.7103               | 0.3151              | 29.0428               | 57.2838                | 0.1348              |
| G10    | Entry no-20     | 8.1312                | 2.3065               | 0.1999              | 18.7108               | 69.8911                | 0.1703              |
| G11    | Entry no-21     | 6.3434                | 4.6143               | 0.2715              | 28.2267               | 59.0254                | 0.1348              |
| G12    | Entry no-22     | 6.4245                | 6.2167               | 0.14689             | 37.2898               | 47.7176                | 0.4276              |
| G13    | Entry no-23     | 8.2963                | 1.2503               | 0.2705              | 33.1717               | 55.9953                | 0.0987              |
| G14    | Entry no-P-S-2  | 6.0635                | 2.5317               | 0.2669              | 38.9009               | 51.3562                | 0.0668              |
| G15    | GP-01002        | 7.4113                | 5.1763               | 0.5016              | 25.3153               | 60.6179                | 0.0020              |
| G16    | GP01004         | 6.5012                | 5.1353               | 0.341               | 40.1636               | 46.7211                | 0.0900              |
| G17    | GP-01005        | 7.4773                | 5.3527               | 0.3678              | 18.1084               | 67.1876                | 0.0591              |
| G18    | GP-01009        | 6.2744                | 6.0924               | 0.3098              | 25.1556               | 60.9614                | 0.2323              |
| G19    | GP-04011        | 8.6344                | 2.4451               | 0.2404              | 26.0249               | 61.9957                | 0.0914              |
| G20    | GP-04012        | 6.3746                | 4.8316               | 0.3228              | 29.6585               | 56.6076                | 0.0923              |
| G21    | GP-04015        | 6.5214                | 3.4655               | 0.2742              | 25.4531               | 63.4574                | 0.0988              |
| G22    | GP-04016        | 6.6776                | 3.4314               | 0.2017              | 23.4684               | 64.9893                | 0.1414              |

**Saturated Fatty Acid**

The hybrids with a high content of saturated fatty acids will have an important impact on the food industry because their oil will permit the production of semi-solid fats without the need of health-detrimental processes such as hydrogenation or trans esterification [2]. But as daily intake for frying oils its percentage should be minimum. Onemli [3], Praveen [4] reported saturated fatty acid exhibit significant variation among their studied germplasm. In this study out of seven fatty acid estimated in parental inbred lines palmitic acid (C16:0), stearic acid (C18:0) and arachidic acid (C20:0) are in group of saturated fatty acid. Palmitic acid (C16:0) ranges 0.17-2.41, stearic acid (C18:0) range 0.30-7.96 and arachidic acid (C20:0) range 0.02-5.52 indicates inbreds are differ from one to another for different kinds of saturated fatty acid (Table 1). Total saturated fatty acid range from 7.95-13.60 (Table 2). Selected inbreds that used for crossing in half diallel fashion it ranges 6.27-8.46 for palmitic acid, 1.25-6.09 for stearic acid and 0.20-0.46 for arachidic acid respectively (Table 3). In crossing generation develop through selected inbreds it ranges 4.58-6.88 for palmitic acid, 1.96-5.17 for stearic acid and 0.20-0.39 for arachidic acid respectively.
| Inbreds   | Percentage of Fatty Acids | Sl no. | Designation | Total saturated fatty acid (SFA) | Total un-saturated fatty Acid (SFA) | Total fatty acid (TFA) |
|-----------|---------------------------|--------|-------------|---------------------------------|-------------------------------------|-----------------------|
| G1        | BHAC-SH-037               | 6.5205 | 5.6109      | 0.2696                          | 17.0873                            | 67.4096               | 0.3181 | 0.2627 |
| G24       | GP-04018                  | 6.3144 | 4.4272      | 0.2929                          | 28.4428                            | 59.4558               | 0.1576 | 0.1299 |
| G25       | GP-04019                  | 5.7923 | 6.1755      | 0.1265                          | 43.6844                            | 42.3745               | 0.4234 | 0.2070 |
| G26       | GP-04023                  | 5.9758 | 3.8181      | 0.266                           | 31.2023                            | 57.1553               | 0.0894 | 0.1048 |
| G27       | GP-04024                  | 6.8573 | 3.9103      | 0.4151                          | 28.0428                            | 55.2838               | 0.1447 | 0.2080 |
| G28       | GP-04026                  | 7.3145 | 5.0245      | 0.3024                          | 19.5954                            | 66.2245               | 0.1585 | 0.7934 |
| G29       | GP-04028                  | 5.2543 | 3.7103      | 0.3798                          | 24.6706                            | 60.8798               | 0.0894 | 0.2070 |
| G30       | GP-04030                  | 6.4347 | 5.4827      | 0.2564                          | 24.2609                            | 62.2216               | 0.1408 | 0.1272 |
| G31       | GP-04038(2)               | 7.9266 | 3.7103      | 0.2618                          | 29.0428                            | 18.3698               | 0.0954 | 0.1545 |

**Table 2:** Total fatty acid composition (Saturated and un-saturated) of the different inbred lines.
Palmitic Acid

Standard sunflower genotypes contain 5-6% palmitic acid. Önenli [3], Praveen [4] observed significant variation for palmitic acid (C16:0) in sunflower genotype. A low level of palmitic acid is preferred from human health point of view. Palmitic acid is believed to increase LDL (low density lipoprotein), which is associated with cardiovascular disease risk. In the present study, the palmitic acid ranges in parental line 5.25-8.63%. Proportion of palmitic acid was low in parental line lines GP-04028 (5.25%) and GP-04019 (5.79%), in selected parent that used for crossing in half diallel fashion lines the lowest recorded was GP-01009 (6.27%) and GP-04026 (7.31%), while in 28 derived crosses, it ranged from 4.58 to 6.88 percent. The lowest proportion of palmitic acid in derived crosses was BHAC-04038(1)xGP-01005 (4.58%), Entry no-20xGP-01004 (4.67%) and it ranges 4.58-6.88 percent. Praveen (2015) reported palmitic acid range 4.43-10.93%. Skoric et al. mentioned palmitic acid may vary 3.0-11.5% and according to Anon. 2015-16 palmitic acid ranges 5-8 percent in sunflower.

Stearic Acid

Stearic acid is categorized as saturated fatty acid, the higher concentrations is an undesirable oil quality characteristic. In case of parental lines, the low proportion was recorded in BHAC-04026 (0.85%), Entry no-23 (1.25%) and BHAC-04038 (1.41%) and stearic acid ranges in parental line 0.85-6.22%, in selected lines for half diallel crosses it was lowest in Entry no-23 (1.25%), BHAC-04038(1) (1.78%) and BHAC-P-S-2 (1.96%) and it ranges 1.25-6.09. While in 28 derived crosses it recorded very low proportion from 1.96 to 5.17 percent. In case of hybrid low proportion was recorded in cross combination GP-01004x Entry-P-S-2 (1.96%), GP-01009x Entry-23 (2.45%) and GP-01004x GP-01005 (3.02%). This result is in accordance with the report of Skoric et al. where they conclude stearic acid may vary 0.6-6.2% in sunflower, Praveen [4] recorded range of stearic acid 0.09-5.27% and stearic acid 4-6 per cent [5].

Arachidic Acid

Arachidic acid is also a saturated fatty acid which found <1% in sunflower. High concentration of this fatty acid is undesirable for human health. FAO/WHO [6] reported arachidic acid is responsible for cardiovascular disease (DVD), coronary heart disease. In this study arachidic acid range in parental line was 0.13-0.53%. The lowest proportion of arachidic acid found in the inbreds GP-04019 (0.13%), Entry no-22 (0.15%) and Entry no-20 (0.20%). In selected parent those were used in crossing programme in half diallel fashion ranges 0.20-0.46%. In derived 28 F1 arachidic acid ranges from 0.20-0.39%. The lowest proportion of arachidic acid in derived F1 was found in the cross combination GP-01004x GP-01005 (0.1959%), GP-01009x Entry-23 (0.2093%), GP-01004x Entry-23 (0.2135%) and GP-01004x Entry P-S-2 (0.2178%).

Unsaturated Fatty Acid (UFA)

The sunflower oil had more than 90% of the unsaturated fatty acids and variation was noted among parents as well as hybrids.
regarding un-saturated fatty acid profile. Among unsaturated fatty acids, the linoleic and oleic is dominant in classical sunflower. There is an important genetic variation regarding the fatty acid composition of the sunflower oil. [7,8]. In this study UFA varied 47.67-91.68 percent which found in the inbred line GP-04038(2) and BHAC-04038(1) respectively. The inbred lines BHAC-04028 (91.0535%), Entry no.P-S-2(1) (90.4485%), BHAC-Sh-037 (90.3701%) and Sun-W-103 (90.1699) contain more than 90% unsaturated fatty acid. Other inbreed contain 80-90% unsaturated fatty acid except only one inbred GP-04038(2) which contain 47.66% UFA. Kostik and Bauer; Skoric et al. [9] reported sunflower oil contain the highest percentage of long chain mono and polyunsaturated fatty acids than other vegetables oil sources. According to FAO/WHO [6] UFA especially Poly Unsaturated Fatty Acid (PUFAs) is essential on human health in the prevention of particularly, cardiovascular disease (DVD), coronary heart disease.

**Oleic Acid**

High proportion of the essential fatty acid (18:2) is considered to reduce blood cholesterol and hence sunflower has a special significance [10]. It has greater oxidative stability and is useful as frying oil in the preparation of snack foods. High oleic acid also explains significant variation for oleic acid while investigating fatty acid profile in sunflower oil. Kostik and Bauer [9] reported high oleic acid (Table 4) respectively. Onemli (2012), Praveen (2015) explain significant variation for oleic acid while investigating fatty acid profile in sunflower oil. Kostik and Bauer [9] reported oleic acid ranges 27-36 percent, Rosa (2014) found oleic acid range 20-25%, Praveen [4] observed oleic acid 24.63-84.97% in their study.

**Table 4:** Fatty acid profile of F1 generation of selected eight parents developed through half diallel fashion.

| Entry | Saturated Fatty Acids (SFA) | Unsaturation Fatty Acid (USFA) |
|-------|---------------------------|--------------------------------|
| Entry | Palmitic Acid (C16:0) | Stearic Acid (C18:0) | Arachidic Acid (C20:0) | Oleic Acid (C18:1) | Linoleic Acid (C18:2) | Linolenic Acid (C18:3) | Cis-ecosenoic Acid (20:1) |
| Entry no.-20 x BHAC-04038(1) | 6.4547 | 3.367 | 0.2773 | 29.977 | 58.9471 | 0.0757 | 0.1525 |
| Entry no.-20x GP-01004 | 4.6996 | 3.813 | 0.3166 | 47.9099 | 42.2706 | 0.0772 | 0.1536 |
| Entry no.-20x GP-01005 | 6.0676 | 3.6139 | 0.2955 | 34.0137 | 54.9263 | 0.0922 | 0.1400 |
| Entry no.-20x GP-01009 | 5.4604 | 3.1893 | 0.2734 | 53.3699 | 36.5458 | 0.0997 | 0.2205 |
| Entry no.-20x GP-04026 | 6.3626 | 4.1047 | 0.293 | 37.5991 | 50.36 | 0.0837 | 0.1244 |
| Entry no.-20x Entry-23 | 6.5916 | 4.2828 | 0.2288 | 23.9345 | 64.0861 | 0.1079 | 0.1090 |
| Entry no.-20 Entry P-S-2 | 5.7898 | 4.6149 | 0.2443 | 34.5122 | 53.9819 | 0.0755 | 0.0889 |
| BHAC-04038(1) x GP-01004 | 5.8682 | 3.7117 | 0.2749 | 35.1755 | 53.9188 | 0.0978 | 0.1427 |
| BHAC-04038(1) x GP-01005 | 4.581 | 3.089 | 0.2744 | 56.8591 | 34.2852 | 0.0010 | 0.1656 |
| BHAC-04038(1) x GP-01009 | 5.0218 | 5.1661 | 0.3908 | 52.0538 | 35.5439 | 0.1535 | 0.2027 |
| BHAC-04038(1) x GP-04026 | 5.7854 | 3.7338 | 0.245 | 34.8341 | 54.6678 | 0.0434 | 0.0949 |
| BHAC-04038(1) x Entry-23 | 5.586 | 3.0349 | 0.2388 | 24.4293 | 65.8399 | 0.0878 | 0.0946 |
| BHAC-04038(1) x Entry P-S-2 | 5.8802 | 4.1271 | 0.232 | 38.7072 | 50.1929 | 0.0831 | 0.2320 |
| GP-01004x GP-01005 | 6.3964 | 3.023 | 0.1959 | 15.845 | 73.7622 | 0.0866 | 0.1344 |
| GP-01004x GP-01009 | 6.5095 | 5.0203 | 0.3753 | 25.1674 | 61.8882 | 0.0642 | 0.0985 |
| GP-01004x GP-04026 | 5.4795 | 3.4541 | 0.2618 | 35.363 | 54.2463 | 0.0751 | 0.1628 |
| GP-01004x Entry-23 | 6.8521 | 3.67 | 0.2135 | 20.73 | 67.8152 | 0.1148 | 0.1102 |
| GP-01004x Entry P-S-2 | 5.8558 | 1.9563 | 0.2178 | 39.7321 | 51.3652 | 0.0917 | 0.1497 |
| GP-01005x GP-01009 | 5.4077 | 3.0269 | 0.2896 | 32.8015 | 57.3424 | 0.0709 | 0.0010 |
| GP-01005x GP-04026 | 6.293 | 3.9712 | 0.2833 | 24.0317 | 64.4185 | 0.079 | 0.1218 |
Linoleic Acid

In traditional sunflower oil, the linoleic acid content will be generally very high (60-70%). Linoleic acid and its derivative fatty acids are essential fatty acids and not synthesized by human beings and hence, must be obtained from dietary sources. High level of linoleic acids in the oil reduces the blood cholesterol level and plays an important role in preventing atherosclerosis [11]. Thus, edible oil with high linoleic acid content is premium oil. According to results of this study Entry no-20 had highest linoleic acid (69.89%) among the studied inbreds which can be used for genetic analysis and breeding programs. Among 31 inbreds line linoleic acid ranges from 18.37-69.89. Others inbred GP-01005 (67.81%), GP-04017 (67.40%) and GP-04012 (67.34%) also contain high percent of linoleic acid which can be utilized in quality breeding to develop healthy sunflower oil. In crossing generation of selected line linoleic acid ranges 31.39-73.76%. Among derived crosses, the highest proportion of linoleic acid recorded in combination GP-01004xGP-01005 (73.76%), GP-01004x Entry-23 (67.81%), BHAC-04038(1)xEntry-23 (65.84%) and GP-01005xGP-04026 (64.42%) compare to their respective parents. Whereas involving parents GP-04026, GP-01009, Entry-23, Entry P-S-2 and GP-01005 contain 0.001-0.79 percent. The selected genotype entry no-21(1.00%) and Entry no-22(1.00%) contain the highest percentages of cis-ecosenoic acid in parental inbred. Selected germplasm it ranges 0.0004-1.00 percent. The selected inbred GP-04026 (0.1585%), GP-04018 (0.1576%), GP-04028 (0.1480%) and GP-04024 (0.1447%) also contain high percent of linolenic acid which can be utilized in quality breeding to develop healthy sunflower oil. In selected lines it ranges 0.06-0.23. In F1 obtain from selected parents it ranges 0.01-0.02%. Among derived crosses, the highest proportion of linolenic acid recorded in combination GP-01005xEntry P-S-2 (0.20%), Entry-23xEntry P-S-2 (0.20%), GP-01009x GP-04026 (0.18%) and GP-04026x Entry P-S-2 (0.15%) respectively. Whereas involving parents GP-04026, GP-01009, Entry-23, Entry P-S-2 and GP-01005 contain 0.0591%, 0.0668%, 0.0987%, 0.2323% and 0.1585% respectively (Table 3). Onemli [3], Praveen [4] reported significant variation for linolenic (C18:3) acid in their study.

Linolenic Acid

It is worthy to note that linolenic acid is also an essential fatty acid; however, its presence in the oil may cause rancidity and off-flavor. In traditional sunflower oil, the linolenic acid content will be generally <1 percent. As like as linoleic acid, linolenic acid and its derivative fatty acids are essential fatty acids and not synthesized by human being and hence, must be obtained from dietary sources. In this study the inbred Entry no-22 and GP-04019 had highest linolenic acid 0.4276% and 0.4234% among the studied lines which can be used for genetic analysis and breeding programs. Others inbred GP-04017 (0.3181%), GP-01009 (0.2323), GP-04026 (0.1585%), GP-04018 (0.1576%), GP-04028 (0.1480%) and GP-04024 (0.1447%) also contain high percent of linolenic acid which can be utilized in quality breeding to develop healthy sunflower oil. In F1 obtain from selected parents it ranges 0.01-0.02%. Among derived crosses, the highest proportion of linolenic acid recorded in combination GP-01005xEntry P-S-2 (0.20%), Entry-23xEntry P-S-2 (0.20%), GP-01009x GP-04026 (0.18%) and GP-04026x Entry P-S-2 (0.15%) respectively. Whereas involving parents GP-04026, GP-01009, Entry-23, Entry P-S-2 and GP-01005 contain 0.0591%, 0.0668%, 0.0987%, 0.2323% and 0.1585% respectively (Table 3). Onemli [3], Praveen [4] reported significant variation for linolenic (C18:3) acid in their study.

Cis-ecosenoic Acid

Cis-ecosenoic acid is also unsaturated fatty acid which found in sunflower genotype <1 percent. Onemli [3], Praveen [4] reported significant variation for cis-ecosenoic acids (C20:1) in their study. In parental line in this study its range is 0.0004-1.00 percent. The genotype entry no-21(1.00%) and Entry no-22(1.00%) contain the highest percentages of cis-ecosenoic acid in parental inbred. Selected germplasm it ranges 0.001-0.79 percent. The selected inbred GP-04026 (0.79%) contain the highest proportion of cis-ecosenoic acid. In derived crosses it ranges 0.01-0.25%. The highest proportion of cis-ecosenoic acid found in the cross combination GP-01009x GP-04026 (0.25%), BHAC-04038(1)x Entry P-S-2 (0.2320%), Entry no-20x GP-01009 (0.2205%) and GP-01005x Entry P-S-2 (0.2124%) respectively [13-21].

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