A CALCULATOR PROGRAMME FOR FACTORIAL EXPERIMENTS

THIS programme can be used to analyse data from a factorial experiment in 3 factors A, B and C at levels l, m and n respectively replicated p times laid out in RBD or CRD using any machine having specifications given by Abdurazak[1]. Codes have been used to identify the various numbers in the print out. The codes used are Ø, 1, 2, 12, 3, 13, 23, 123 and 11 respectively for replication, A, B, AB, C, AC, BC, ABC and error. Many characters recorded from the same experiment can be processed. Each character has to be assigned a reference number to recognise its output in the print out. The codes and reference number are printed with a negative sign. The name of the design is also to be supplied in the form of a code; Ø for RBD and 1 for CRD. Two factor experiments can be analysed by putting $n = 1$, and simple RBD/CRD by putting $m = 1$ and $n = 1$. If $r = 1$, the highest order interaction will be taken for error. The total number of data registers should be greater than or equal to $l + m + n + p + lm + ln + mn + 38$. All inputs are printed and spaces provided at appropriate places.

Input

(*a*) Parameters : Design code, p, l, m, n, Student's t at df for error. (*b*) Reference no. (*c*) The data in the order $a_1b_1c_1r_1$, $a_1b_1c_1r_2, \cdots$ $a_1b_1c_2r_1, \cdots a_1b_2c_1r_1 \cdots a_2b_1c_1r_1 \cdots a_1b_mc_nr_p$.

Output

Grand total, total SS ; means for replications; marginal and two factor combination means in the order A, B, AB, C, AC, BC ; CD for these means except replications; the SS, df, MS and F for replications, A, B, AB, C, AC, BC, ABC and error respectively.

The programme is given below :

Name of the programme : FACTORIAL

```
000  AG4 HP T1
007  HP S1Ø + 37 = SØ31
020  1 S1 S11 TØ T2
030  11 T3 21 T4 31 T9
042  R2 SØ HP T8
050  1 T5 1Ø T6 2Ø T7
061  MR9 I9 + MR2 = SR9
074  I2 I3 I4 I6 I7
084  MR7 × 1Ø + RØ = SR4
097  MR5 × R8 = SR2 I5
109  R8 − 1 × MR6 = SR3
121  MØ E R5 ≤ Ø61
130  IØ 3 E RØ ≤ 042
140  M1Ø S1 − 1 × S11
151  (M8 − 1) = S19
161  M1 × M8 = S9 =
170  Ø S38 E R1 ≤ 192
181  M11 FØ19 Ø S11
192  MØ19 HP SØ2Ø
202  Ø SØ21 11 SØ29
213  GØ HA ± P M32 T2
224  M34 T4 M36 T6
234  Ø S3Ø D31 S1Ø
244  R6 T9 M33 T3 M37 T7
258  R9 T6 M35 T5
267  38 T1 Ø SØ = 1 T8
278  HP FØ FR1 × = F3Ø I1
292  I8 M1 E R8 ≤ 278
303  MØ F31 FR2 FR3 FR4
317  FR5 FR6 FR7 × = F1Ø
331  I5 I6 I7
337  R5 + 1 = E M36 E ≤ 267
351  I3 I4 R3 + 1 =
360  E M34 E ≤ 258
369  AI2 R2 + 1 =
377  E M33 E ≤ 244
386  M31P × = ÷ M9 = SØ ±
399  F3Ø M3Ø PA 11 T7
411  38TØ T1 1 T2 21 T8
424  MR7 E 1 T5 ≤ 461
435  Ø SRØ IØ I5
443  MR2 E R5 ≤ 435
453  Ø SR1 → 524
461  MR8 ± P M9 ÷ MR2 = T4
475  MRØ ÷ R4 = P
483  C ZRØ × = FR1 IØ
494  I5 MR2 E R5 ≤ 475
506  MR1 ÷ R4 − MØ = SR1
519  AR7 T9
524  I1 I2 I7 I8
532  7 E R2 ≤ 424
540  Ø E MR7 ≤ 553
549  R7 T9
553  I7 19 E R7 ≤ 54Ø
564  M4Ø + M42 = ± F44
576  M39 + M42 = ± F43
588  M1Ø ÷ M1 − MØ −
598  S46 M41 − M42 −
609  M43 − M44 = S45
620  M39 + M4Ø = ± FØ41
633  Ø E M19 ≤ 657
642  M3Ø − M46 − M38 = S46
657  R9 + 27 = T8
665  MR8 ÷ MR9 = SØ1Ø
677  2TØ 12T1
684  Ø E MR1 ≤ 713
693  M1Ø × 2 × MRØ ÷ M9 =
706  √ × M2Ø = P
713  IØ I1 7 E RØ ≤ 684
725  A38 TØ 21 T1 11 T3
738  Ø E MR3 ≤ 769
747  MR1 ± P
752  MRØ P ÷ MR3 P ÷ P
```

763 M1Ø = PA
769 Ø SRØ IØ I1 I3
779 19 E R3 ≤ 738
788 AA → 213
794 Halt.

Department of Statistics,
College of Agriculture,
Vellayani, Trivandrum 695 522,
India, *July* 23, 1979.

M. P. ABDURAZAK.

1. Abdurazak, M. P., *Curr. Sci.*, 1980, **49**, 3.
2. Panse, V. G. and Sukhatme, P. V., *Statistical Methods for Agricultural Workers*, Indian Council of Agricultural Research, New Delhi, 1961.

CHOLESTEROL AND BIOSYNTHESIS OF DIOSGENIN BY TUBER-CALLUS OF *DIOSCOREA DELTOIDEA*

SAPOGENINS, including diosgenin, have been reported from tissue cultures of a number of *Dioscorea* species and some other plants[1]. Cholesterol, a steroid precursor and a key intermediate in the biosynthesis of diosgenin has been shown, by using 4-C-^{14} and 26-C-^{14} cholesterol, to be incorporated in diosgenin through callus cultures of *D. deltoidea*[2]. Effect of cholesterol on diosgenin production by seedling-callus of *D. deltoidea* has been studied in detail[3]. However, they[3] reported a decrease in diosgenin production when the cultures were initially fed with cholesterol. On the other hand, initially fed cholesterol has been found to remarkably increase diosgenin production by callus cultures of *Trigonella foenum-graecum*[4] and *D. floribunda, Costus speciosus, Solanum aviculare* and *S. xanthocarpum*[5]. The present authors therefore examined the effect of initially fed cholesterol on diosgenin biosynthesis by tuber-callus of *D. deltoidea*, and the results are reported in this communication.

Callus of young tuber tissue of *Dioscorea deltoidea* Wall., kept on rapidly proliferating (*ca.* 25-fold increase in fresh weight during 50 days) on a modified nutrient agar medium of Schenk and Hildbrandt[6], for more than 2 years, was used as the inoculum. Composition of the culture medium in mg/l, where it differed from SH medium[6] was: 500 NH_4NO_3, 500 meso-inositol, 2 indoleacetic acid (IAA) and 0·5 kinetin (Kn); whereas, 2,4-dichlorophenoxy-acetic acid (2,4–D) remained at the original conc. of 0·5 mg/l. Cholesterol prepared in hot ethanol was added to the medium at 50, 100, 150 and 200 mg/l conc. before autoclaving. Ten replicate cultures of each treatment were incubated under 3 klx fluorescent light for 15 hr daily and at 27° ± 1° C. Sterilization procedure and other cultural conditions were as reported earlier[7].

Callus grown on different treatments was harvested after 60 days of incubation, dried and analysed separately for diosgenin content. Each of the dried tissue samples was refluxed with 5% (v/v) HCl in 70% ethanol for 4 hr. Mixture was filtered and the residual tissue was soxhleted with chloroform and estimated for diosgenin[8].

The acetylated isolated compound gave mp 194°–195° C, the mmp remained undepressed and its ir spectra were superimposable with that of standard diosgenin acetate, all of which confirmed that the compound was diosgenin.

In the control, without any supplement of cholesterol, the diosgenin content was minimum being 1·03%. However, 1·03% diosgenin in tuber-callus is nearly double the content (0·682%) previously reported by Chaturvedi and Srivastava[7] from the same tissue. This increase in diosgenin content is attributable to the composition of the changed nutrient medium. Diosgenin content of callus increased with all levels of cholesterol as compared to the control except at its lowest conc. of 50 mg/l in which the amount of diosgenin remained unchanged. Maximum diosgenin, *i.e.*, 1·88% was biosynthesised at 100 mg/l cholestrol whereas at its higher conc. of 150 and 200 mg/l the diosgenin content of callus progressively decreased being 1·57% and 1·13%, respectively. The present results are in conformity with the reports of stimulatory effect of initially fed cholesterol on diosgenin production by callus cultures of *Trigonella*[4] as also of *D. floribunda, C. speciosus* and *Solanum* spp[5]., but not with the results of Kaul *et al.*[3]. Also, this stimulatory effect of cholesterol is not affected by autoclaving (*cf.* Khanna *et al.*[4]). Addition of cholesterol at any conc. used in the present study did not inhibit callus growth to any appreciable extent. Such observations find support in the results of the studies on tissue culture of *Digitalis mertonensis*[9] where cholesterol promoted callus growth. On the contrary, in previous investigations on callus cultures of *D. deltoidea*[3] and of *Trigonella*[4] cholesterol inhibited callus growth. That is how, such a high content of diosgenin as 2·58%[3] obtained with the use of a combination of cholesterol and yeast extract has been reported to be nullified by the poor growth of cultures of *D. deltoidea* so much so that there has been "..... no appreciable net gain in diosgenin yield per flask ...."[3]. In the light of such observations, the 1·88%[3] diosgenin obtained in the present study by feeding 100 mg/l cholesterol accompanied by prolific growth of callus appears to be quite significant.