

## Research Article

# Genetic study of resistance to downy mildew in muskmelon (*Cucumis melo* L.)

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### Abstract

Thirty F<sub>1</sub> hybrids were developed by crossing six female parents with five male parents in a line x tester mating design. Adult muskmelon plants of parents and hybrids were evaluated in greenhouse and field conditions. Significant variances for general combining ability (GCA) and specific combining ability (SCA) indicated the importance of both additive and non-additive gene actions for the expression of resistance. Inbred lines IIHR 352, IIHR 190 and IIHR 122 demonstrated consistently high and negative *gca* effects in both screening conditions. Arka Jeet x IIHR 121, Arka Jeet x IIHR 122, Punjab Sunehri x IIHR 190, Punjab Sunehri x IIHR 718, IIHR 681 x IIHR 121, IIHR 681 x IIHR 122 and IIHR 352 x IIHR 616 were the hybrids expressed high *per se* performance, high mid-parent heterosis and high *sca* effects. The study highlights the importance of harnessing useful genes from diverse parental lines to improve resistance to *Pseudoperonospora cubensis* in adapted muskmelon varieties/cultivars.

### Key words

Muskmelon, heterosis, combining ability, per cent disease index, adult plant resistance, downy mildew

### Introduction

Worldwide, downy mildew [*Pseudoperonospora cubensis* (B & C) Rost.] is the most serious disease in muskmelon (*Cucumis melo* L.). In India, vulnerability of muskmelon to foliar diseases is well known, particularly downy mildew is responsible for heavy loss in fruit yield. The disease occurs mainly on leaves, which reduces the photosynthetic capability of the muskmelon plants.

Currently, resistant varieties or hybrids are the most economical means to control downy mildew in muskmelon. Inoculation methods for downy mildew (Thomas, 1982) have demonstrated to be useful for detecting differences between muskmelon genotypes. Although the adult plant stage is usually the important stage for resistance screening, often seedlings were assessed for disease reactions in controlled conditions. Cohen *et al.* (1984) had shown large positive correlations between seedlings and adult plant reactions and between greenhouse and field disease responses, whereas, Perchepied *et al.* (2005) used two variables: the disease score at the final scoring date and the area under the disease progress curve (AUDPC) to assess the disease in seedlings and adult plants in muskmelon.

There are several reports on inheritance of downy mildew resistance (Cohen *et al.*, 1985; Epinat and Pitrat, 1989; Epinat and Pitrat, 1994 a&b; Kenigsbuch and Cohen, 1992; Thomas *et al.*, 1988) in muskmelon. But there are no published studies available on combining abilities and heterosis for adult-plant resistance to downy mildew in muskmelon. Combining abilities helps in assessing the lines used as parents in the

production of superior hybrid combination. Quantitative genetic data on resistance is valuable for identification of those single crosses and parents with high resistance. Therefore, the present study was conducted with the following objectives: (1) to identify parents and specific combinations of parents expressing resistance to downy mildew in a set of female and male parents, (2) to estimate the heterosis for resistance in F<sub>1</sub> hybrids to downy mildew, (3) to estimate genetic parameters useful for describing the mode of inheritance of host resistance to muskmelon downy mildew.

### Materials and methods

**Plant materials:** A set of six female lines *viz.*, Arka Jeet, Punjab Sunehri, RM 43, *ms-1*, IIHR 681, IIHR 352 and five male parents (testers) included IIHR 616, IIHR 190, IIHR 718, IIHR 121 and IIHR 122 were crossed in a line x tester fashion to derive thirty F<sub>1</sub> hybrids. Inbred line IIHR 352 (*C. melo* var. *reticulatus*) is highly resistant to powdery mildew and downy mildew diseases. IIHR 190 (*C. melo* var. *reticulatus*), IIHR 121 and IIHR 122 are resistant while IIHR 718 is moderately resistant to downy mildew. Arka Jeet, Punjab Sunehri, RM 43, *ms-1*, IIHR 681 (*C. callosus*) and IIHR 616 were susceptible to downy mildew. None of these lines specifically bred for race specific resistance to *P. cubensis*.

**Experimental plot:** Seedlings were raised in 50 unit plastic potting trays inside greenhouse. At 2–3 leaf stage, seedlings were transplanted to the main field on raised beds at the Vegetable Block, Indian Institute of Horticultural Research, Bangalore. The experiment was laid out during third week of February 2006. To take observation individual

seedling was spaced at 3.0 m between beds (centre to centre) and 0.45 m within bed. Field plots with 15 plants each arranged in three randomized blocks. The each parent and F<sub>1</sub> hybrid planted one plot per block.

**Inoculation in greenhouse:** Parents and hybrids were raised in 50 unit potting trays containing sterilized coco-peat as growth media in the greenhouse in September 2006. For each parent and cross, 10 plants each in were raised in three replicates. Inoculation and post inoculation procedure as proposed by Cohen *et al.* (1984) and Kenigsbuch and Cohen (1992). The source pathogen was isolated from a muskmelon growers' field near Bangalore, India. A colony of *Pseudoperonospora cubensis* maintained on susceptible variety, Arka Jeet in greenhouse at 18–26°C. Infected leaves from Arka Jeet were collected and gently washed in distilled water to release the spores. Test plants were inoculated on ad axial leaf surfaces with sporangial suspension containing 10,000 sporangia per milliliter using atomizer. The concentration of spores was measured with a hemocytometer. The inoculated plants were kept in high humidity black polythene tent for about 20 h and returned to greenhouse bench. On the seventh night, seedlings were again placed in high humidity black polythene tent for 20 h to allow fungal sporulation. Disease reactions were noticed on 8th day after inoculation. Plants were maintained for 6 weeks after inoculation. Seedlings were hand watered every day. Nutrition solution containing 150 mg N, 150 mg P and 150 mg K per liter of water was supplied every week. One spray of micronutrients @ 0.5 ml/l of water was supplied at 2–3 leaf stage. Seedlings trays were arranged with proper spacing on greenhouse benches to allow the spread of growing plants.

**Inoculation in field:** Seedlings of all the parents and hybrids were raised in 50 unit plastic potting trays containing sterilized coco-peat as growth media in greenhouse. At two-leaf stage, seedlings were transplanted to main field in November 2006. Seven days after transplanting, the ad axial leaf surface was sprayed with a sporangial suspension containing 10,000 sporangia per milliliter by hand sprayer. Susceptible variety Arka Jeet was planted at regular intervals all over the field for uniform spread of disease. Seedlings were spaced at 3.0 m between beds (centre to centre) and 0.45 m within bed. Field plots with 10 plants each were arranged in three randomized blocks. The parents and crosses were planted one plot per block.

**Disease assessment:** Cohen *et al.* (1984) used percent leaf loss to describe the reaction of older plants in field plots and correlated lesion type in artificial inoculation at 2-leaf stage in greenhouse to facilitate the selection of resistant plants. Perchepped *et al.* (2005) used two variables: the

disease score at the final scoring date and the area under the disease progress curve (AUDPC) to assess the disease in seedlings and adult plants. In the present study, disease was assessed 30 days after inoculation, which coincides with the flowering stage in the greenhouse experiment and 50 days after inoculation, which coincides with fruit development stage in field experiment. Plants in greenhouse were assessed at the flowering stage (30 days after inoculation) for understanding of resistance of adult plants. Each plant was visually assessed for percent leaf area infected, using linear 0 to 5 scale indicating average grade of all the leaves. 0 = healthy and no symptoms, 1 = 1–5%, 2 = 6–10%, 3 = 11–20%, 4 = 21–30%, 5 = 30% of total leaf area covered with chlorotic and/or necrotic symptoms. The Percent Disease Index (PDI) was calculated using the formula proposed by Wheeler (1969):  $PDI = \text{Sum of numerical values} / (\text{number of leaves graded} \times \text{maximum rating}) \times 100$ .

**Statistical analysis:** The data were analysed as per the line x tester method suggested by Kempthorne (1957) using the model proposed by Arunachalam (1974) and described by Singh and Choudhary (1985). Using expected mean sum of squares, the formulae for covariance of half sibs and full sibs that in turn give the variances because of GCA and SCA. The sum squares for genotypes were subdivided into variation among parents, variation among parents *vs.* hybrids and variation among hybrids. The sum of squares for parents was subdivided into variation among lines x testers, variation among lines and variation among testers.

Mid-parent heterosis (MPH) and high-parent heterosis (HPH) were estimated as the percentage deviation of the F<sub>1</sub> mean from the mid-parent (MP) and high-parent (HP) values, respectively.

$$\text{MPH (\%)} = 100 \times (F_1 - \text{MP})/\text{MP}$$
$$\text{HPH (\%)} = 100 \times (F_1 - \text{HP})/\text{HP}$$

Simple correlation coefficients were calculated between parents and progeny. GCA of all 11 parents was correlated to mean PDI of all parents to calculate GCA–mean PDI of parent correlation. Mean PDI of all 30 hybrids was correlated to mid-parental values of all thirty hybrids to calculate F<sub>1</sub> hybrid–mid-parent correlation.

## Results and discussion

Analysis of variance detected large parental diversity and manifestation of genetic variability in their hybrids as suggested by highly significant mean sum of squares due to parents, parent *vs.* hybrid and hybrids. The partitioning of mean sum of squares revealed the variances due to parents, lines, testers, hybrids, parents *vs.* hybrids and line x testers were also significant (Table 1). Mean sum of squares due to the parent *vs.* hybrid was statistically significant indicating that average mid-

parent heterosis was significant. Non-significant replication mean sum of squares in the greenhouse and field conditions suggested that infection was uniform in both the screening experiments. Variances due to GCA and SCA for mean PDI were significant indicating the importance of additive and non-additive gene action for the inheritance of resistance to downy mildew in both screening conditions.

Mean PDI of parents varied from 3.72 to 70.47 and 8.80 to 65.64 in the greenhouse and in the field conditions, respectively (Table 2). Mean PDI of hybrids varied from 8.32 to 44.67 and 8.48 to 39.60 in artificial and field conditions, respectively (Table 2). Disease assessment based on percent-leaf-area-infected found useful for evaluating adult-plant resistance in field as well as the greenhouse conditions. The results obtained in two different screening experiments were comparable.

Estimates of general and specific combining ability effects are presented in table 2. Negative GCA effects detected in parental lines indicated greater resistance, and large positive GCA effects indicated greater susceptibility. Parents Punjab Sunehri, IIHR 352, IIHR 190, IIHR 121 and IIHR 122 were good general combiners for downy mildew resistance (Table 2). Many hybrids (Arka Jeet x IIHR 121, Arka Jeet x IIHR 122, Punjab Sunehri x IIHR 616, Punjab Sunehri x IIHR 718, IIHR 681 x IIHR 121, IIHR 681 x IIHR 122, IIHR 352 x IIHR 616 and IIHR 352 x IIHR 718) involving one of the parents as good general combiner, expressed significantly negative SCA effects indicating the presence of non-additive gene action (Table 2). Hybrids (Arka Jeet x IIHR 718, RM 43 x IIHR 718 and *ms-1* x IIHR 616) involving both parents with positive GCA effects expressed significantly negative SCA (poor x poor combination of parents) effects, which implied additive x additive gene effects and complementary gene action (Table 3). In the susceptible parent Punjab Sunehri, genes for resistance to downy mildew are expressed only in combinations indicating one or more complementary gene exist in Punjab Sunehri. Hybrid IIHR 352 x IIHR 190 with both parents with negative GCA effects expressed negative SCA effects indicating additive x additive gene effects and duplicate type of gene action.

Twenty hybrids expressed significant and negative mid-parent heterosis in both experiments (Table 3). The mid-parent heterosis varied from -74.13 (Arka Jeet x IIHR 122) to 103.80 (IIHR 352 x IIHR 190) in greenhouse and varied from -77.22 (Arka Jeet x IIHR 122) to 156.53 (IIHR 352 x IIHR 121) in field experiment (Table 3). There was tendency towards higher resistance than either parent in some crosses. The hybrids had tendency to higher resistance than mid-parent value in many hybrids

as indicated by a negative mid-parent heterosis (negative dominance). In hybrids where one or both of the resistant parents were involved, there was better resistance than their mid-parent value. This may be an effect of different resistance genes in the parents. HPH varied from -50.15 to 875.78 percent in the greenhouse experiment and varied from -57.70 to 359.30 per cent in the field experiment (Table 3). Hybrids Punjab Sunehri x IIHR 616, Punjab Sunehri x IIHR 718 and RM 43 x IIHR 718 expressed high parent heterosis in the desirable direction indicating true heterosis which can be explained by genetic complementation between the parents. High parent heterosis of -50.15 % in greenhouse experiment and -57.70 % in field experiment implied considerable potential for further increasing the resistance by a systematic search of heterotic groups.

The most important finding of the present study is the identification of three lines, namely 'IIHR 352' (*C. melo* var. *reticulatus*), IIHR 190 (*C. melo* var. *reticulatus*) and IIHR 122 demonstrated consistently high and negative GCA effects in the greenhouse as well as in field conditions. These lines, therefore, have potential for use in developing broad-sense resistance to downy mildew. The results in the present study highlight the importance of harnessing useful genes from diverse parental lines to improve resistance to *P. cubensis* in adapted varieties/cultivars. The hybrids obtained from these diverse parents expressed high heterosis which implied diverse parents could produce higher resistance.

The performance of parents appears to be a useful indicator of the parent of their hybrids for resistance breeding with high mid-parent heterosis and close correlation between hybrid *per se* and mid-parent values (Table 4). Quantitative genetic theory states that heterosis is a function of genetic diversity between parents (Falconer, 1989). The strong correlation between GCA and parents *per se* performance suggested that the performance of *per se* could be a good indicator of its ability to transmit the resistance to its progenies (Table 4). The GCA effects are reliable as the results obtained are based on data recorded on the performance of parents and combination of parents in hybrids in two different screening experiments. However, maximum gain from selection of parents can be achieved from test-crossing.

Both additive and non-additive gene effects found important with preponderance of non-additive gene effects for resistance to downy mildew therefore, some forms of recurrent selection like diallel selective mating (Jensen, 1970) or biparental mating in early segregating generations might prove to be effective approach. Biparental progeny selection (Andrus, 1963) might be used to get transgressive segregants from hybrids involving

good x good and poor x good combination of parents. Such hybrids could be promising for isolation of superior recombinants for downy mildew resistance in advanced generations of segregation. Exploitation of hybrid vigour for downy mildew resistance could be achieved through hybrids Arka Jeet x IIHR 121, Arka Jeet x IIHR 122, Punjab Sunehri x IIHR 190, Punjab Sunehri x IIHR 718, IIHR 681 x IIHR 121, IIHR 681 x IIHR 122 and IIHR 352 x IIHR 616 with high *per se* performance, mid-parent heterosis and SCA effects. These promising hybrids involved one or both the parents with good GCA effects.

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**Table 1. Analysis of variance for percent disease index (PDI) of downy mildew in artificial and field conditions in muskmelon**

| Sources                         | df | Greenhouse Experiment | Field Experiment |
|---------------------------------|----|-----------------------|------------------|
| Treatment                       | 40 | 744.23**              | 767.08**         |
| Replication                     | 2  | 2.94                  | 5.94             |
| Parents                         | 10 | 1737.49**             | 1784.79**        |
| Lines                           | 5  | 534.12**              | 579.13**         |
| Testers                         | 4  | 808.21**              | 672.61**         |
| Line x Testers                  | 20 | 170.95**              | 138.39**         |
| Hybrids                         | 29 | 310.01**              | 279.01**         |
| Hybrids vs. Parents             | 1  | 4568.08**             | 6166.34**        |
| Error                           | 80 | 2.58                  | 2.11             |
| $\sigma^2$ GCA                  | -  | 27.08**               | 27.79**          |
| $\sigma^2$ SCA                  | -  | 45.43**               | 56.12**          |
| $\sigma^2$ GCA : $\sigma^2$ SCA | -  | 1 : 1.68              | 1 : 2.02         |

\*, \*\* significant at 5 and 1 per cent level, respectively

**Table 2. Estimates of general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of hybrids and *per se* (PDI) for resistance to downy mildew in greenhouse and field conditions in muskmelon**

|               | Female (SCA effects) |          |          |          |          | Male     |         |               |
|---------------|----------------------|----------|----------|----------|----------|----------|---------|---------------|
|               | Arka Jeet            | PS       | RM 43    | ms-1     | IIHR 681 | IIHR 352 | GCA     | <i>Per se</i> |
| <b>Male</b>   |                      |          |          |          |          |          |         |               |
| IIHR 616      | 9.37**               | -1.36    | -2.29**  | -3.73**  | 0.61     | -2.87**  | 5.24**  | 49.26(S)      |
|               | 9.84**               | -1.74*   | -1.86*   | -3.35**  | -0.53    | -1.47*   | 5.35**  | 25.55(MR)     |
| IIHR 718      | 1.88                 | -10.08** | -3.81**  | 3.92**   | 10.85**  | -1.03    | 7.53**  | 32.97(MR)     |
|               | 3.71**               | -8.83**  | -4.03**  | 1.48     | 10.84**  | -2.98**  | 6.52**  | 36.15(MR)     |
| IIHR 190      | 3.07**               | -8.06**  | 11.32**  | -11.03** | 2.65**   | -2.87**  | -1.10** | 3.72(R)       |
|               | -1.60                | -6.29**  | 10.27**  | -9.02**  | 3.31**   | -1.15    | -1.78** | 12.32(R)      |
| IIHR 121      | -7.11**              | 16.06**  | -4.48    | 0.71     | -8.17**  | 3.60**   | -5.35** | 18.30(R)      |
|               | -6.82**              | 11.94**  | 0.20     | 0.16     | -6.96**  | 2.44**   | -3.82** | 5.60(R)       |
| IIHR 122      | -7.20**              | 3.44**   | -0.73    | 10.13**  | -5.94**  | 3.18**   | -6.32** | 12.88(R)      |
|               | -5.13**              | 4.92**   | -4.58**  | 10.73**  | -6.67*   | 3.16**   | -6.26** | 8.80(R)       |
| <b>Female</b> |                      |          |          |          |          |          |         |               |
| GCA           | 0.46                 | -3.14**  | 2.23**   | 6.84**   | 2.47**   | -11.53** |         |               |
|               | -0.62                | -2.88**  | 3.69**   | 6.51**   | 1.81**   | -12.24** |         |               |
| <i>Per se</i> | 63.13(S)             | 42.14(S) | 70.47(S) | 51.69(S) | 49.73(S) | 4.44(R)  |         |               |
|               | 65.64(S)             | 42.13(S) | 57.53(S) | 45.82(S) | 54.98(S) | 7.56(R)  |         |               |

\*, \*\* significant at 5 and 1 per cent level, respectively

Upper lines and lower lines represent values in the greenhouse and field experiments, respectively.

Letters in the parentheses represent the reaction of the each genotype against powdery mildew inoculation: R, resistant; MR, moderately resistant; S, susceptible.



**Table 3. Mid-parent heterosis (MPH), high parent heterosis (HPH) of thirty F<sub>1</sub> hybrids evaluated in green house and field conditions for resistance to downy mildew in muskmelon**

| Hybrids              | Greenhouse condition |                |          |          | Field condition |                |          |          |
|----------------------|----------------------|----------------|----------|----------|-----------------|----------------|----------|----------|
|                      | MP                   | F <sub>1</sub> | MPH      | HPH      | MP              | F <sub>1</sub> | MPH      | HPH      |
| Arka Jeet x IIHR 616 | 56.20                | 37.97          | -32.44** | -22.93** | 45.60           | 35.07          | -23.08** | 37.26**  |
| Arka Jeet x IIHR 718 | 48.05                | 32.77          | -31.81** | -0.61    | 40.32           | 30.10          | -40.86** | -16.70** |
| Arka Jeet x IIHR 190 | 33.43                | 25.33          | -24.23** | 581.43** | 41.54           | 17.37          | -55.44** | 40.99**  |
| Arka Jeet x IIHR 121 | 40.72                | 10.90          | -73.23** | 71.20**  | 35.69           | 9.23           | -74.09** | 64.82*   |
| Arka Jeet x IIHR 122 | 38.01                | 9.83           | -74.13** | -15.23   | 40.27           | 8.48           | -77.22** | -3.64    |
| PS x IIHR 616        | 45.70                | 24.56          | -46.26** | -50.15** | 16.56           | 21.22          | -47.36** | -16.90** |
| PS x IIHR 718        | 37.55                | 18.13          | -51.71** | -44.99** | 50.90           | 15.30          | -66.46** | -57.70** |
| PS x IIHR 190        | 22.93                | 11.52          | -49.76** | 209.87** | 45.62           | 9.53           | -71.72** | -22.60*  |
| PS x IIHR 121        | 30.22                | 31.39          | 3.89     | 393.04** | 46.84           | 25.72          | -15.23** | 359.30** |
| PS x IIHR 122        | 27.51                | 9.87           | -64.12** | -14.91   | 40.99           | 16.27          | -49.06** | 84.89**  |
| RM 43 x IIHR 616     | 59.87                | 29.00          | -51.56** | -41.13** | 45.57           | 27.67          | -33.39** | 8.30     |
| RM 43 x IIHR 718     | 51.72                | 29.77          | -42.44** | -9.71*   | 21.86           | 26.67          | -43.06** | -26.20** |
| RM 43 x IIHR 190     | 37.10                | 36.27          | -2.23    | 875.78** | 38.98           | 32.67          | -6.46*   | 165.20** |
| RM 43 x IIHR 121     | 44.39                | 18.30          | -58.77** | 187.43** | 33.70           | 20.00          | -34.90** | 267.00** |
| RM 43 x IIHR 122     | 41.68                | 19.00          | -54.42** | 63.76**  | 34.93           | 13.33          | -59.81** | 51.48**  |
| ms-1 x IIHR 616      | 50.48                | 32.17          | -36.27** | -34.70** | 29.07           | 29.00          | -18.73** | 13.50*   |
| ms-1 x IIHR 718      | 42.33                | 42.10          | -0.54    | 27.71**  | 33.65           | 35.00          | -14.60** | -3.18    |
| ms-1 x IIHR 190      | 27.71                | 18.53          | -33.11** | 398.57** | 9.94            | 16.19          | -44.31** | 31.41**  |
| ms-1 x IIHR 121      | 35.00                | 26.01          | -25.67** | 308.53** | 35.62           | 23.34          | -9.22*   | 316.80** |
| ms-1 x IIHR 122      | 32.29                | 34.47          | 6.76     | 197.13** | 30.34           | 31.47          | 15.23**  | 257.60** |
| IIHR 681 x IIHR 616  | 49.50                | 32.13          | -35.08** | -34.77** | 31.57           | 27.13          | -32.62** | 6.18     |
| IIHR 681 x IIHR 718  | 41.35                | 44.67          | 8.03**   | 35.49**  | 25.71           | 39.67          | -12.94** | 9.74*    |
| IIHR 681 x IIHR 190  | 26.73                | 27.83          | 4.15     | 648.88** | 30.29           | 23.83          | -29.18** | 93.43**  |
| IIHR 681 x IIHR 121  | 34.02                | 14.43          | -57.58** | 126.65** | 6.58            | 11.52          | -61.97** | 105.70** |
| IIHR 681 x IIHR 122  | 31.31                | 18.88          | -39.69** | 62.76**  | 37.22           | 9.37           | -70.62** | 6.48     |
| IIHR 352 x IIHR 616  | 26.85                | 14.65          | -45.43** | 229.78** | 31.94           | 12.13          | -26.73** | -52.50** |
| IIHR 352 x IIHR 718  | 18.71                | 18.78          | 0.42     | 322.73** | 33.17           | 17.78          | -18.65** | -50.80** |
| IIHR 352 x IIHR 190  | 4.08                 | 8.32           | 103.80** | 87.17**  | 27.31           | 10.32          | 3.79     | -16.30** |
| IIHR 352 x IIHR 121  | 11.37                | 10.53          | -7.37    | 137.06** | 31.89           | 16.88          | 156.53** | 201.40** |
| IIHR 352 x IIHR 122  | 8.66                 | 9.15           | 5.60     | 105.85** | 8.18            | 20.00          | 146.30** | 128.90** |

\*, \*\* significant at 5 and 1 per cent level, respectively; MP = Mid-parent, HP = High parent

**Table 4. Some important phenotypic correlation coefficient between parent and progeny for resistance to downy mildew in greenhouse and field conditions**

| Combination                     | Correlation coefficient |                 |
|---------------------------------|-------------------------|-----------------|
|                                 | Greenhouse condition    | Field condition |
| GCA-Parent <i>per se</i>        | 0.65**                  | 0.53**          |
| Mid-parent-Hybrid <i>per se</i> | 0.61**                  | 0.43**          |

\*\* Significant at 1 per cent level