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Phenotyping tobacco recombinant inbred lines for solanesol

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Abstract
Solanesol is a pharmaceutically important phytochemical found in tobacco. In order to hasten the process of tobacco cultivar development for solanesol production, isolation and mapping of major genes responsible for production of solanesol, an immortal mapping population was developed. The study was undertaken to phenotype the Recombinant Inbred lines (RIL) population for solanesol for understanding the genetics nature of solanesol and identifying high solanesol recombinants. A total number of 260 RILs developed from the cross between HDBRG, a high solanesol line and By-53, a low solanesol line along with parents were phenotyped for solanesol content for five years (2012-17) and statistically analysed. The solanesol varied from 4.90% to 0.05% in different years. While, average solanesol varied in different years from 1.01% (2012-13) to 2.41% (2014-15) with an average of 1.73% for five years. The mean solanesol content in the RILs found to be between 0.91% and 2.88% during 2012-17 with 2.15% HDBRG and 1.34% By-53. Various statistical parameters estimated indicated that solanesol content is normally distributed among the population with trangressive segregation which shows that it is polygenic and controlled by many genes with minor and influenced by environment. A total of 21 genotypes with higher solanesol content than HDBRG parent were identified.

Keywords: tobacco, recombinant inbred lines, RILs, solanesol, mapping population, phenotyping

Introduction
Solanesol ($C_{45}H_{74}O$) is a ubiquitous compound present in plant kingdom, especially in family Solanaceae (Taylor and Fraser, 2011; Campbell, 2016; Yan *et al*., 2015) [25, 3, 29]. Ever since Rowland *et al.* (1956) [17] isolated solanesol from tobacco plant, considerable literature has been generated on its chemical extraction and varied uses. Solanesol found to have high commercial use in production of valuable pharmaceutical compounds. Solanesol is the starting material for synthesis of co enzyme Q9, co enzyme Q10, Vitamin K2, Vitamin E and N-solanesyl-N, N′-bis(3,4-dimethoxybenzyl) ethylenediamine (SDB) (Colowick and Kaplan, 1975; Campbell *et al*., 2016 [3]; Parmar *et al*., 2015 [16] and Yan *et al*., 2015) [29]. Coenzyme Q10 has the potential for the treatment of migraines, neurodegenerative diseases, hypertension, and cardiovascular diseases in view of its anti-oxidant and anti-aging properties and is also reported to strengthen the body's immune system, cardiovascular function and improve brain health (Bentinger *et al*., 2010; Parmar *et al*., 2015; Sarmiento *et al*., 2016; Yan *et al*., 2015) [1, 16, 18, 29], and it is also being used as a dietary supplement by patients with type 2 diabetes (Mezawa *et al*., 2012) [13]. Vitamin K2 reported to promote bone formation and mineralization, inhibits bone resorption, has preventive and therapeutic effects on osteoporosis, promotes blood coagulation, and improves arterial stiffness (Hamidi *et al*., 2013) [6]. Meanwhile, SDB can plays a synergistic role with certain antitumor drugs and overcome several types of drug resistance in tumours mediated by P-proteins and (Enokida, *et al*., 2002; Sidorova, *et al*., 2002) [4, 22]. Yao *et al*. (2015) [30] also reported that solanesol could protect human hepatic L02 cells from ethanol-induced oxidative injury via upregulation of HO-1 and Hsp70 expression. Thus, the medical benefits of solanesol and its derivatives are well established.
In view of the importance of the solanesol, ICAR-Central Tobacco Research Institute (CTRI), Rajahmundry has identified high solanesol yielding tobacco types/ varieties by GC and HPLC analysis. In order to hasten the process of cultivar development and aids in isolation of major genes responsible for production of solanesol in tobacco, a Recombinant Inbred Line (RIL) population was developed at ICAR-CTRI. RILs are immortal mapping population developed from the F_2 generation through single seed descent.

These are a set of homogeneous and homozygous lines. The advantage with RILs is that the same mapping population can be maintained and used over and over again to map any traits that differs between the parental strains used to generate the population. They can also assist in analysing multiple loci contributing to any trait of interest. The downside is that they are less statistically powerful for analyzing effects of any one particular locus, because each RIL also harbors potentially confounding background genetic variation. The present paper deals with the phenotyping of an RIL population developed for solanesol trait. Such study will help to understand the genetics nature of solanesol and assists in identifying high solanesol recombinants for further use.

Materials and Methods

A total of 260 recombinant Inbred lines developed from the cross between HDBRG, a high solanesol line and By-53, a low solanesol line were raised along with parents at Katheru Farm during 2012-13, 2013-14, 2014-15, 2015-16 and 2016-17. The leaf samples collected from mapping population and their parents at peak flowering stage were air-dried and powdered by following standard procedures. Solanesol was estimated from the ground leaf powder adopting the external standard method using the SIGMA standard (Narasimaharao *et al*., 2000). For Solanesol estimation, the sample was accurately weighed (100 mg) and placed in a 150 ml conical flask with stopper. 25 ml of analytical reagent grade isopropyl alcohol was added to the flask. Contents of the flask were mechanically shaken for 30 minutes, filtered through Whatman No.1 filter paper and the 5 µl filtrate was injected into SCHIMADZU High Performance Liquid Chromatography (HPLC) System, Model LC-8A equipped with a UV-VIS detector at 210 nm, using a INERTSIL ODS-3 reverse-phase column of dimensions 4.6 x 250 mm. The mobile phase was isopropyl alcohol: methanol (60:40) at a flow rate of 1 ml/min. Solanesol was estimated adopting the external standard method using the SIGMA standard (Narasimaharao *et al*., 2000).

The solanesol contents estimated during the season were analysed statistically. Mean, minimum, maximum, standard deviation, coefficient of variance and Standard error of Deviation and RILs having significantly higher Solenesol over mean (No) were estimated for each year. Five years data was statistically analysed for calculation of SEm and CD 5%. Population distribution, empirical cumulative distribution frequency, distribution fitting, normality tests viz., Kolmogorov-Smirnov (Kolmogorov, 1933; Smirnov, 1948) [9, 23], Shapiro-Wilk (Shapiro and Wilk, 1965) [20], Anderson-Darling (Stephens, 1974) [24], Lilliefors (Lilliefors, 1967, 1969) [11, 12] and Jarque-Bera (Jarque and Bera, 1981) [8] and percentile values for the population was calculated using XLSTAT software Version 2014.5.03 (https://www.xlstat.com/en/news/xlstat-2014-5). The population distribution for solanesol was plotted using relative frequency and empirical cumulative distribution frequency. Distribution fitting was done using estimated density and density distribution from the input parameters using the XLSTAT software. Genotypes recording Mean + 2 SEd solanesol values were considered as significantly superior ones. Among the significant ones, lines recording higher solanesol than the high solanesol yielding tobacco lines were identified.

Results

The solanesol content estimated in the 260 RILs for five years (2012-17), varied from a maximum of 4.90% (2014-15 & 2015-16) to minimum of 0.05% (2012-13) (Table 1). While, average solanesol varied in different years from 1.01% (2012-13) to 2.41% (2014-15) with an average of 1.73% for five years. Number of RILs recording significantly higher solanesol over mean ranged from 23 to 119 in different years. All the years, HDBRG recorded higher solanesol than low solanesol parent BY-53 (Table 1). The solanesol content of HDBRG ranged from 1.40 to 2.50% in different years with a mean of 2.15% and BY-53 from 0.60 to 1.70% with the mean of 1.43%.

The mean solanesol content estimated for 2012-17 in the RILs found to be between 0.91% and 2.88% (Table 2) with 2.15% in high solanesol yielding parent, HDBRG and 1.34% in low parent, By-53. Twenty seven RILs (10% of the population) recorded higher solanesol than HDBRG and 30 RILs (12% of the population), lower than By-53. Twenty five percent of the population has above 1.95% solanesol and another 25% of population has below 1.49 (Table 3).

As the variation observed is continuous for the solanesol in the present study, population frequency estimated for intervals of 0.2%. Highest frequency (64 No.) of population found to be in 1.59 to 1.79% with 0.246 relative frequency (Table 4) and lowest frequency in 2.78 to 2.98% with one individual. When the mean solanesol content was plotted against relative frequency (Fig. 1) and the plot of estimated density and density distribution (Table 4) from the input parameter (Fig. 2), both confirmed the normal distribution for solanesol content in the population with zero skewness and. Kolmogorov-Smirnov test also confirmed the normal distribution with 94% probability. The computed p-values for other statistics viz., Shapiro-Wilk, Anderson-Darling, Lilliefors and Jarque-Bera tests that are calculated to test the null hypothesis that data come from a normally distributed population for solanesol in RILs, are greater than the significance level alpha=0.05 indicating the samples drawn from normal distribution (Table 5). As empirical cumulative distribution frequency (ECDF) gives a better estimate of the CDF than a histogram gives of the Population Distribution Frequency (PDF). A 'nonparametric bootstrap' procedure uses the sample ECDF in place of the unknown population CDF. Empirical cumulative distribution for solanesol content (%) in RIL population also showed the normal distribution (Fig 3).

Identification of stable high solanesol yielding lines in the population can be useful in production of solanesol for commercial purpose. Hence, the RILs recording higher solanesol than HDBRG and significantly superior over mean for four (15 No.) and five (6 No.) years were identified and listed (Table 6). In four year category, the mean solanesol ranged from 2.16 to 2.88% and five years from 2.16 to 2.63%. The solanesol content recorded in different RILs identified in five year category is depicted in Fig. 4. The entry 1/135 recorded highest solanesol (2.88%) among the identified lines and 1/179 and 1/241 lowest (2.16%). The genotypes, 1/1 and 1/169 recorded lowest mean solanesol values (0.91%).

Discussion

The solanesol content estimated in the 260 RILs for five years varied from a maximum of 4.90% to minimum of 0.05%. Average solanesol in different years varied from 1.01% to 2.41% with an average of 1.73% for five years. This wide variation of solanesol between years indicates the influence of environment on solanesol synthesis. The relative solanesol values for parents, HDBRG and BY-53 were consistent over years indicating that solanesol is genetically controlled. Zhou

and Liu (2006) [32] also observed a strong influence of environment and/or genetic background on solanesol content in one of the study confined to a single tobacco cultivar, where solanesol content In tobacco leaf samples collected from 16 regions of China ranged from 0.4% to 1.7% dry weight. Kotipalli *et al*., 2008 [10] recorded genotypic variation for leaf solanesol content in several tobacco cultivars with mean values ranged from 0.05% dry weight to 1.77%. Zhao *et al*., 2007 [31] also found that the solanesol contents in different varieties of *Nicotiana tabacum* ('K326', 'Yunyan 85', 'NC89', 'NC82', 'Honghuadajinyuan', 'Piaohe 1' and 'Longjiang 911') and *Nicotiana rustica* ('Hamatou') were different, the content of solanesol in 'Honghuadajinyuan' variety of tobacco was the highest. Burton *et al*. (1989) [2] observed that genotype, growing conditions and agronomic practices have profound influence on solanesol content in the leaf at various growth stages of the plant. They found a fivefold difference in solanesol concentration among genetic lines and the growing season contributed to a tenfold difference of solanesol for certain tobacco genotypes. Soil moisture deficits enhanced solanesol concentration at least fourfold. Irrigation of the stressed tobacco decreased the solanesol level. Solanesol concentration increased dramatically after topping for the top stalk position and there were marginal increases for the bottom and middle stalk positions. Nitrogen fertilization had only a minimal influence on solanesol concentration. Schlotzhauer *et al*., 1989 [19] recorded lower levels of solanesol in closer spacing (1, 11, 000 plants/ha) as against wider spacing (22,000 plants/ha). Solanesol seemed to be concentrated in the "green" chloroplast fraction of the leaf and became associated with the fiber fraction as the leaf matured (Woodlief *et al*., 1984; Narasimha Rao *et al*., 2000) [27, 15]. Total solanesol generally decreased with topping but significantly increased between 60th and 80th day of growth and then increased less rapidly until the 100th day. Solanesol content increased from lower leaves to the upper leaves. Solanesol decreased with nitrogen dose ranging from 60 to 480 kg/ha (Vidal and Tancogne, 1981) [26]. According to Narasimha Rao and Chakraborty (1986) [14], in FCV tobacco, solanesol gradually increased with maturity and reached a maximum value in the cured leaf (immature: traces, mature: 0.25%, over-mature: 0.55%, cured: 1.07%) and a similar trend was observed in the case of bidi tobacco also (immature: 0.25%, mature: 1.25%, cured: 0.60%). Sheen *et al*. (1978) [21] also observed that solanesol increased during leaf growth and reached a maximum at leaf maturation, culminating in an increase of free solanesol due to the hydrolysis of solanesyl esters after curing. Thus, all these studies conclusively proved that the solanesol production in a plant is genetic in nature and influenced by environment and production practices. In contrast to the studies by different researchers for the variation in solanesol in different varieties, the present investigation for the first time dealt with its variation in RILs.

Ten percent of the population (27 RILs) recorded higher solanesol than HDBRG and 12% of the population (30 RILs) lower than By-53 indicating trangressive segregation in the RILs. Xiao *et al.* (1996) [28] also observed transgressive segregants for 13 quantitative traits including grain yield in a recombinant inbred population derived from sub-specific rice cross.

Fisher *et al*. (1932) [5] showed that, for a one-locus system, positive average *h* makes the frequency distribution of F_2 plants skewed to the left, indicating that desirable alleles are dominant. On the other hand, negative average *h* produces rightward skewness with the undesirable alleles being dominant. When there is no additive epistasis, skewness of the frequency distribution is equal to zero, but is greater than or smaller than zero in the presence of complementary or duplicate interaction, respectively. In RILs dominance and epistasis cannot be measured because no heterozygotes are available. However, in RIL population, both dominant and co-dominant traits segregate into 1:1 ratio due to absence of heterozygotes. Also, members of a fixed mapping population will contain differing amounts of recombination and linkage disequilibrium between loci will be present. RILs on the other hand go through many rounds of recombination before becoming fixed. In case of qualitative characters the population falls into discrete categories. Absence of such discreteness and continuous variation for the trait in the population indicates the character is controlled by many genes with small effects. Normal distribution for solanesol content in the population indicates that the solanesol content is polygenic and controlled by many genes and zero skewness and kurtosis indicates lack of epistasis and linkage. Hence, Quantitative Trait Loci (QTLs) needs to be identified for mapping the solanesol genes.

The present study identified stable high solanesol yielding lines in the population. These entries can be used in the production of solanesol for commercial purpose or can be used as parents in breeding programmes. The solanesol yields in these RILs can further be maximised through the development of suitable agro-techniques.

Table 1: Description statistics for solanesol content (%) in RILs during 2012-13 to 2016-17

Year	RILs							HDBRG	BY-53
	Mean	Maximum	Minimum	SD	CV%	SEd	No. of RILs having significantly higher Solenesol over mean		
2012-13	1.01	2.63	0.05	0.52	52.07	0.033	23	2.15	1.34
2013-14	1.41	3.25	0.25	0.57	40.51	0.035	107	2.40	1.65
2014-15	2.41	4.90	0.50	0.77	31.99	0.048	119	2.50	1.40
2015-16	1.75	4.90	0.50	0.71	40.40	0.044	97	2.30	1.70
2016-17	2.06	4.10	0.50	0.65	31.57	0.040	112	1.40	0.60
2012-17 (Average)	1.73	2.88	0.91	0.34	19.72	0.021	114	2.15	1.34

Table 2: Mean solanesol content (%) in RILs and parents (2012-17)

Genotype	Sol.
1/1	0.91
1/2	1.65
1/3	1.23
1/4	1.18
1/5	1.71
1/6	1.49
1/7	1.47
1/8	2.17
1/9	1.81
1/10	1.15
1/11	1.30
1/12	1.43
1/13	1.30
1/14	1.22
1/15	1.37
1/16	1.39
1/17	1.63
1/18	2.03
1/19	1.70
1/20	2.28
1/21	1.92
1/22	1.49
1/23	1.87
1/24	1.36
1/25	0.93
1/26	1.15
1/27	1.06
1/28	1.40
1/29	1.82
1/30	1.49
1/31	1.62
1/32	1.55
1/33	1.50
1/34	2.56
1/35	2.01
1/36	2.29
1/37	1.71
1/38	2.09
1/39	1.59
1/40	1.63
1/41	2.08
1/42	1.37
1/43	1.24
1/44	1.40
Genotype	**Sol.**
1/45	2.09
1/46	1.48
1/47	1.52
1/48	1.94
1/49	2.48
1/50	1.39
1/51	1.78
1/52	1.93
1/53	1.47
1/54	1.67
1/55	2.10
1/56	1.78
1/57	1.72
1/58	1.73
1/59	2.11
1/60	2.02
1/61	1.69
1/62	2.23
1/63	1.39
1/64	1.61
1/65	1.72
1/66	1.44
1/67	1.11
1/68	1.49
1/69	2.05
1/70	1.65
1/71	1.47
1/72	2.01
1/73	1.97
1/74	1.49
1/75	1.78
1/76	1.90
1/77	2.59
1/78	2.35
1/79	2.11
1/80	1.70
1/81	2.01
1/82	1.25
1/83	1.26
1/84	1.37
1/85	1.83
1/86	1.89
1/87	1.40
1/88	1.65
Genotype	**Sol.**
1/89	1.31
1/90	1.77
1/91	1.31
1/92	1.91
1/93	1.57
1/94	1.09
1/95	1.78
1/96	1.43
1/97	1.95
1/98	1.93
1/99	1.95
1/100	2.03
1/101	1.97
1/102	1.63
1/103	1.78
1/104	1.98
1/105	1.03
1/106	1.09
1/107	1.54
1/108	1.72
1/109	1.64
1/110	2.14
1/111	1.18
1/112	1.70
1/113	1.69
1/114	2.14
1/115	1.62
1/116	1.49
1/117	1.78
1/118	2.03
1/119	1.80
1/120	1.62
1/121	2.11
1/122	1.86
1/123	1.93
1/124	1.85
1/125	1.44
1/126	1.99
1/127	1.60
1/128	1.65
1/129	1.49
1/130	1.71
1/131	1.65
1/132	1.56

Genotype	Sol.
1/133	1.44
1/134	1.58
1/135	2.88
1/136	1.94
1/137	1.53
1/138	1.72
1/139	1.49
1/140	1.08
1/141	1.74
1/142	1.77
1/143	1.58
1/144	2.47
1/145	1.61
1/146	1.97
1/147	1.76
1/148	1.24
1/149	1.25
1/150	1.60
1/151	1.52
1/152	1.79
1/153	1.61
1/154	1.70
1/155	1.87
1/156	1.10
1/157	1.83
1/158	1.51
1/159	1.83
1/160	2.28
1/161	1.46
1/162	1.86
1/163	1.48
1/164	1.42
1/165	1.94
1/166	1.80
1/167	2.63
1/168	1.16
1/169	0.91
1/170	1.93
1/171	1.75
1/172	1.78
1/173	2.20
1/174	2.18
1/175	2.07
1/176	2.17
Genotype	**Sol.**
1/177	1.82
1/178	2.36
1/179	2.16
1/180	2.34
1/181	1.41
1/182	1.46
1/183	1.91
1/184	1.67
1/185	1.71
1/186	2.36
1/187	2.01
1/188	1.69
1/189	1.96
1/190	1.83
1/191	1.52
1/192	1.73
1/193	2.03
1/194	1.60
1/195	1.56
1/196	2.04
1/197	2.07
1/198	1.64
1/199	1.74
1/200	2.06
1/201	1.92
1/202	1.48
1/203	1.79
1/204	1.53
1/205	1.97
1/206	2.01
1/207	1.40
1/208	1.45
1/209	1.47
1/210	1.41
1/211	1.24
1/212	2.17
1/213	1.74
1/214	2.14
1/215	1.80
1/216	1.86
1/217	1.42
1/218	1.81
1/219	1.58
1/220	2.18
Genotype	**Sol.**
1/221	1.82
1/222	1.72
1/223	2.03
1/224	1.82
1/225	1.53
1/226	1.98
1/227	1.66
1/228	1.65
1/229	1.75
1/230	1.63
1/231	1.27
1/232	1.55
1/233	1.71
1/234	1.95
1/235	1.89
1/236	1.87
1/237	2.25
1/238	2.63
1/239	1.89
1/240	1.98
1/241	2.16
1/242	2.43
1/243	1.98
1/244	1.63
1/245	2.42
1/246	2.06
1/247	2.19
1/248	2.04
1/249	1.87
1/250	1.29
1/251	1.77
1/252	1.49
1/253	1.56
1/254	1.69
1/255	1.57
1/256	1.72
1/257	1.58
1/258	1.28
1/259	1.41
1/260	1.63
HDBGR	2.15
By-53	1.34

Table 3: The Percentile of RILs and their solanesol values

RILs Percentile	Solanesol %
Maximum 100%	2.880
99%	2.604
95%	2.290
90%	2.160
3rd Quartile 75%	1.950
Median 50%	1.710
1st Quartile 25%	1.490
10%	1.290
5%	1.160
1%	0.922
Minimum 0%	0.910

Table 4: Descriptive statistics for the intervals of solanesol (%) content in RILs

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0.60	0.79	0	0.000	0.000	0.003
0.79	0.99	3	0.012	0.058	0.012
0.99	1.19	12	0.046	0.232	0.042
1.19	1.39	22	0.085	0.426	0.103
1.39	1.59	53	0.204	1.026	0.181
1.59	1.79	64	0.246	1.239	0.228
1.79	1.99	50	0.192	0.968	0.206
1.99	2.19	36	0.138	0.697	0.134
2.19	2.38	11	0.042	0.213	0.063
2.38	2.58	5	0.019	0.097	0.021
2.58	2.78	3	0.012	0.058	0.005
2.78	2.98	1	0.004	0.019	0.001

Table 5: Estimated statistics on the input data and computed using the estimated parameters of the Normal distribution for solanesol in RILs

Statistic	Data	Parameter
Mean	1.728	1.728
Variance	0.116	0.116
Skewness (Pearson)	0.246	0.000
Kurtosis (Pearson)	0.169	0.000
Normality test	**p-value**	
Kolmogorov-Smirnov test	0.937*	
Shapiro-Wilk	0.393*	
Anderson-Darling	0.601*	
Lilliefors	0.705*	
Jarque-Bera	0.217*	

Table 6: Lines with higher solanesol than HDBRG having significantly superior than mean for more than 4 years

S. No	Genotype	2012-13	2013-14	2014-15	2015-16	2016-17	2012-17
Superior for 5 years							
1.	1/135	1.5	1.6	4.9	3.6	2.8	2.88
2.	1/167	2.63	2.1	2.3	3.5	2.6	2.63
3.	1/34	1.6	1.9	3.3	3.2	2.8	2.56
4.	1/20	1.2	2.2	2.5	2.3	3.2	2.28
5.	1/160	1.3	1.6	3.5	2.3	2.7	2.28
6.	1/179	2.4	1.5	2.1	1.9	2.9	2.16
Superior for 4 years							
7.	1/238	2.63	1.80	3.50	3.30	1.90	2.63
8.	1/77	0.80	1.85	3.80	3.30	3.20	2.59
9.	1/49	1.50	3.10	3.70	2.30	1.80	2.48
10.	1/144	1.10	1.45	4.10	3.10	2.60	2.47
11.	1/242	2.43	1.90	4.20	0.80	2.80	2.43
12.	1/245	1.40	3.00	3.70	1.20	2.80	2.42
13.	1/178	2.10	2.00	3.00	2.70	2.00	2.36
14.	1/36	1.60	1.65	1.80	3.80	2.60	2.29
15.	1/237	0.70	2.35	3.60	2.40	2.20	2.25
16.	1/173	1.50	1.00	3.30	2.20	3.00	2.20
17.	1/247	1.00	2.25	2.90	2.20	2.60	2.19
18.	1/174	1.90	2.00	3.50	1.00	2.50	2.18
19.	1/212	1.90	0.25	2.90	1.90	3.90	2.17
20.	1/8	1.20	1.45	2.60	3.00	2.60	2.17
21.	1/241	1.50	2.20	3.30	0.80	3.00	2.16
Mean+2SED		1.08	1.48	2.51	1.84	2.14	1.77

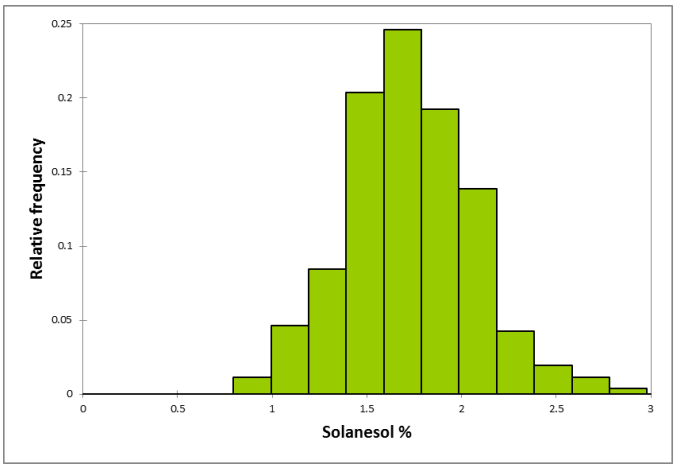

Fig 1: Frequency distribution for solanesol content in RILs (2012-17)

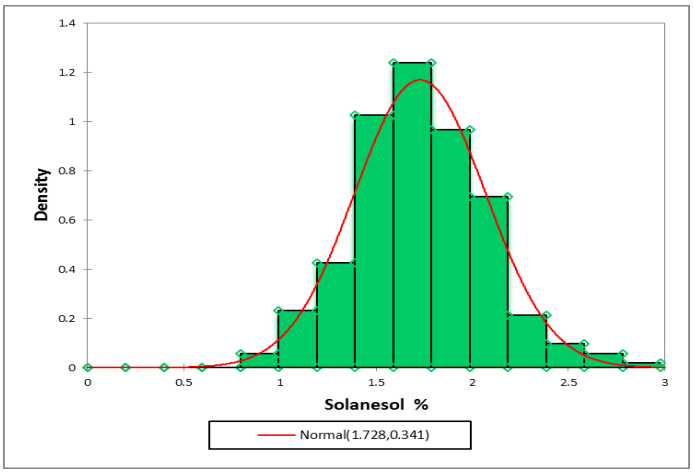

Fig 2: Estimated density and density distribution of solanesol content in RILs (2012-17)

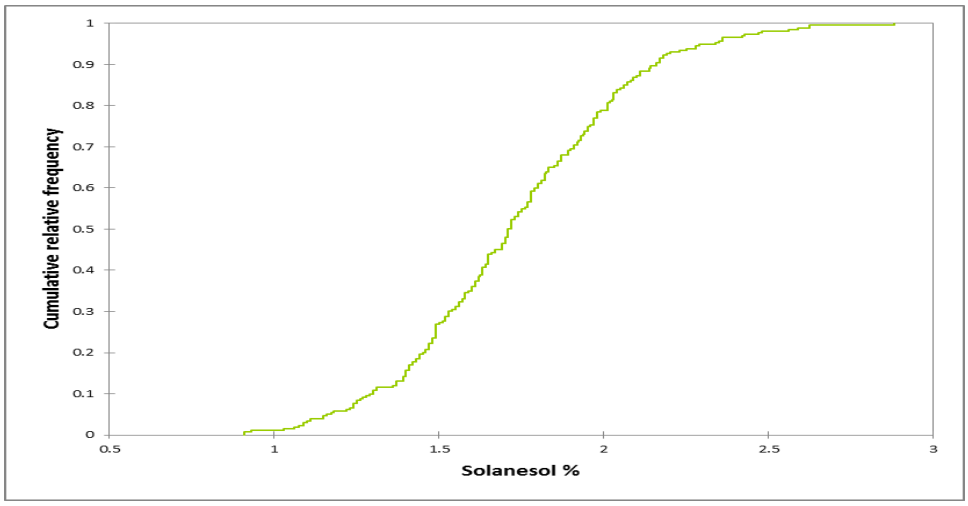

Fig 3: Empirical cumulative distribution for solanesol content (%) in RIL population (2012-17)

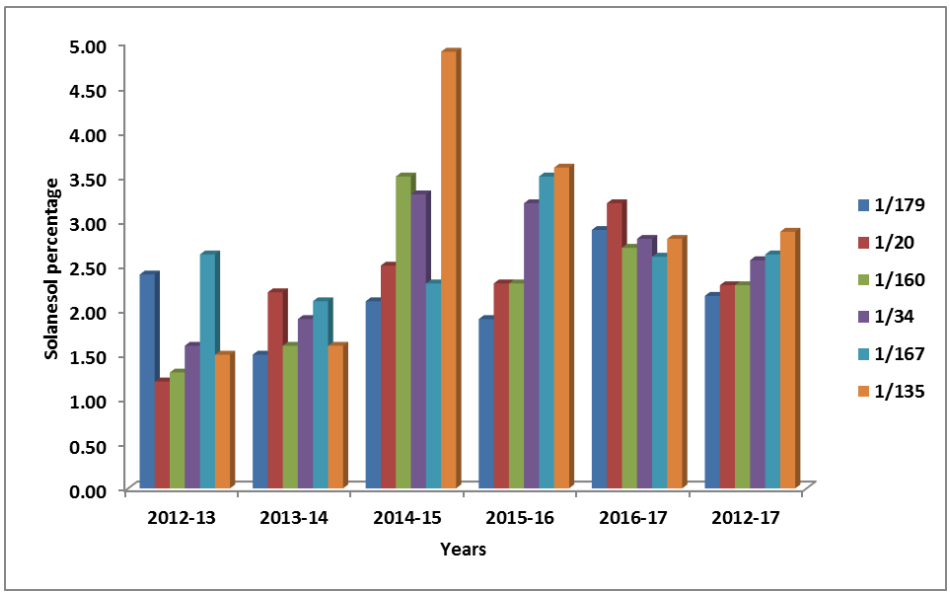

Fig 4: Genotypes with significantly higher solanesol percent than mean for five years (2012-17)

Conclusion

Thus, the results indicate that solanesol is a quantitative character and influenced by environment. The information generated can be effectively co-related in identifying and mapping of QTLs for solanesol, once the genotypic data of the population is available. The identified lines can either be directly used for solanesol production or in breeding programmes for the development of high solanesol lines.

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