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Research Note

Relationship between morphological traits and secondary metabolites in *Artemisia annua* L. by using correlation and path analysis

Shilpi Paul, A A Naqvi, Madan M Gupta, Suman P S Khanuja
Central Institute of Medicinal and Aromatic Plants (CSIR), Lucknow, India
E-mail:shilpipaul01@gmail.com

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Abstract:
Correlation and path analysis were performed for fifteen morphological and chemical (monoterpenes and sequeterpenes) traits in *Artemisia annua*. Significant positive relationship and direct effect was observed of artemisinin yield with plant height, branching pattern (number of primary, secondary and tertiary branches), petiole length, ketone and 1, 8 cineol while negative association was observed with artemisinic acid, α-pinene and camphor. Oil content was negatively associated with leaf characters namely lamina length, lamina width and petiole length. Oil has negative effect on artemisinin content. The information can be used as a useful reference for elucidating relationship of Artemisinin (sesquiterpene), essential oil (monoterpene) with morphological characters for improvement of agronomic practices and will be helpful in selecting superior genotypes.

Key words: *Artemisia annua*, correlation, path analysis, artemisinin, essential oil

Artemisia annua L. (Asteraceae; 2n=18) through scientific intervention in several laboratories has emerged as one of the most promising medicinal plant, yielding phyto-compound 'artemisinin' to fight the deadly infectious disease malaria in the developed and underdeveloped countries of the world, which is caused by the protozoan microbe *Plasmodium* species (Klayman *et al*., 1984; WHO, 2000). This plant is native to China, but is now found widely growing in countries like India, Argentina, Bulgaria, France, Spain, USA, and the former Yugoslavia (Klayman, 1989, 1993) and there have been reports of significant variation in artemisinin content in different ecotypes of *A. annua* found in different locations (Delabays *et al*., 1993). The artemisinin yield in plants of *A. annua* is quite low, ranging from 0.1 to 1% of the plant dry weight, depending on the geographical origin of the plant (Wallaart *et al*., 1999; Khanuja *et al*., 2008; Paul *et al*., 2010). Artemisinin content varies with age of plant, photoperiod and nutrient etc.; highest artemisinin content was reported before flowering (Delabays *et al*., 1993) however, a lot of research is going on to enhance the artemisinin production through multiharvest (Kumar *et al*., (2004) and salinity stress etc (Qian *et al*., 2007) .

A. annua is terpene rich plant, the essential oils and artemisinin being associated with secretary cells based on the association of mono- and sesqui-terpenes with well-defined secondary structure (Croteau, 1986; Woerdenbag *et al*., 1993; Bhakuni *et al*., 2001). The highly aromatic volatile oil having ketone, 1-8 cineol, camphor, germaerene-D, camphene hydrate, α-pinene, β- caryophllyne, myrcene and artemisia-alcohol have been reported to be present in *A. annua*. Low yield of these compound especially artemisinin may increase the demand of this plant.

Yield is a complex trait, depending upon a large number of characters which are often multigenic/polygenic in nature and are highly affected by environmental factors (Nadarajan and Gunasekaran, 2005). Therefore, genetics of traits governing yield and its components may be is useful to understand the prepotency of the lines to select the parents of potential genetic constitution. Hence for developing an efficient selection index, elucidation of interrelationship between yield and its components is imperative and their mutual association of plant characters, which is determined by correlation coefficient relationships. In correlation analysis values of two characters are analyzed on a paired basis, results of which may be either positive or negative. This can be used to find out the degree (strength) of mutual relationship between various plant characters and the component character on which selection can be relied upon for realizing genetic improvement of yield.

Path coefficient analysis is a standardized partial regression coefficient that allows partitioning of correlation coefficient into direct and indirect effects of number of traits towards dependent variable, and also helps in assessing the cause-effect relationship as well as effective selection (Singh and Narayanam, 2007). The concept of path analysis was developed by Wright (1921) and Dewery & Lu (1959) have used this technique in plant selection. This analysis plays an important role in determining the degree of relationship between yield and its components.

The basic goal of breeding for active ingredient is to increase the yield and establish the genetic associationship between the agro morpho and chemical characters. *A. annua* is a highly open pollinated species (Ferriera *et al*., 1995a), considering the highly cross pollinated nature of this plant, correlation and path analysis have been carried out up to four cycles and effectiveness of agro-morpho and chemical characters like oil, oil components (monoterpenes) and artemisinin (sesqueterpenes) measured.

Basic plant material was collected from Kashmir (Sharma *et al*., 1991) and ten seedlots were selected and cultivated for the study up to four cycles in CIMAP Lucknow for the evaluation and breeding purpose of artemisinin and the experimental methodology was followed as described in Paul *et al* (2010). In each cycle high yielding genotypes (based on their artemisinin content) were selected and planted in randomized block design in three replications with a distance of 50X30 cm^2 and supplemented with 20:40:40 kg/ha of N:P:K at the time of planting. The artemisinin yield and its association with agro morphological, oil and oil components were determined by evaluating the plants up to four cycles. Individual plants were harvested for estimation of artemisinin and oil in the month of June, temp 45 ± 2°C. Morphological and chemical data were recorded each plot in each replication for fifteen quantitative characters viz., plant height, number of primary, secondary, tertiary branches, petiole length, lamina length, lamina width, artemisinin, artemisinic acid, oil, α-pinene, β-pinene, camphor, ketone and 1-8 cineol. Estimates of correlation coefficient and path analysis were done following (Dewey and Lu., 1959) through standard statistical methods using SYSTAT software (version 10). The simple correlation coefficients between all possible combinations of variable was worked out by Pearson correlation analysis method to determine relation between traits, especially, such of those which were related to yield. The path- coefficient technique was performed to recognize direct and indirect effects of traits which depend on the artemisinin yield.

Highly significant and positive correlation was observed between plant height with number of primary branches (0.605), secondary branches (0.422), tertiary branches (0.788), lamina width (0.402), petiole length (0.258), artemisinin (0.60) and 1-8 cineole, but on the contrary the relationship between plant height with artemisininc acid (-0.198), α-pinene (-0.207) and camphor (-0.385) was highly significant and negative. Similar result was also observed in number of primary, secondary, tertiary branches (Table-1). These finding seems logic because our field data showed that increasing the plant density in terms of plant height, number of primary, secondary and tertiary branches increased yield of artemisinin. These results are in line with some other research in *Carum copticum* (Ajowan) and sweet fennel (Singh and Mittal 2003; Cosge et al., 2009; Dalkani et al., 2011) in which yield increased with increase in the number of umbrella and plant height. Furthermore, there was highly significant and positive correlation between lamina width and artemisinin and ketone. There was highly significant and positive correlation between the key component artemisinin yield with the plant height (0.64), number of primary branches (0.567), secondary branches (0.474), tertiary branches (0.705), leaf width (0.551), petiol length (0.428), ketone (0.147) and 1-8 cineole (0.478) while strong negative correlation with artemisininc acid (-0.194), α pinine (-0.164) and camphor (-0.307). This result seems true because artemisinic acid is precursor of artemisinin, when the key metabolite is increased, the precursor will decrease. These results are in harmony with those obtained by Liao *et al*., (2009); Chen et al., (2010) and Lui *et al*., (2010). Similar result was also reported by Mishra *et al*., (2010) in chilli, where capsaicin content is positively related with number of branches. It is obvious that leaf character is positively associated with artemisinin because artemisinin synthesis was reported in trichome which is found in aerial part of plant mainly in leaf and flowers.

The artemisinic acid is positively related with ketone. Oil is negatively associated with leaf characters. Oil component like α and β- pinene and ketone is positively associated with oil. Similarly, Habib *et al*., (2007) reported highest correlation of plant height with number of leaves per plant, followed by head diameter and stem diameter. When these all correlation data was converted into dendrogram camphor was separated from rest of the traits and all the morphological traits along with the oil and artemisinin were in one group and oil components were in another group except 1,8 cineol (Fig 1).

To determine the relative importance, the trait data were subjected to path analysis. It permits the separation of correlation coefficient with component of direct and indirect effect. After getting information from the results of correlation analysis, the path coefficient analysis was done to determine direct and indirect effects of traits on artemisinin content. The traits namely primary, secondary and tertiary branches, lamina width, petiole length showed significant ($p<0.01$ and $p<0.05$) and positive genetic association with artemisinin content. Plant height, number of primary branches, secondary branches and tertiary branches showed high direct effect on artemisinin. This result is in line with the report of Liu *et al*., (2010). Number of tertiary branches (r=0.919) showed high degree of direct effect which is followed by plant height (r=0.837), number of primary (0.746) and secondary branches (r=0.629), leaf width (r=0.725) and petiole length (0.571) whereas oil (r=-0.95), artemisinic acid (r=-0.212), α-pinene (-0.174) camphor (-0.354) and 1,8 cineol (r=0.631) showed negative impact on artemsinin. Similarly, Patil *et al*., (1996) has reported positive direct effect of number of seeds per head on seed yields in sunflower. Similar results were observed in our study where artemisinin yield depends on total herb characters like plant height, number of primary, secondary and tertiary branches, petiole length and 1, 8 cineol. Misra *et al*. (2010) also reported similar result in chilli where number of primary branches directly affects capsaicin content. In this context Raiker *et al*., (2005) and Farhad *et al*., (2008) also found similar result.

In *A. annua*, the open pollination (Ferriera *et al*. 1995) leads to detectable variation in different artemisinin yielding genotypes like the members of family Asteraceae. The chemical characters such as artemisinin, oil and other components segregate like any other phenotypic characters as multigenic characters always segregate in the progeny population. Due to the heterogenous behavior, the progeny plants with high artemisinin content may not yield same amount of the chemical component. Therefore, genetic association and their path analysis provide information about the traits which can be used for genetic improvement.

It is concluded that artemisinin yield and oil can be improved by increase in plant density in terms of branching pattern, ketone and 1,8 cineole while oil content will decerase with increase in leaf area. It is also observed that chemical compositions of the essential oils and artemisinin from *A. annua* consistent, although content of each component are prominently different. These differences are largely related with the difference in phytogeographical/environmental factors, their growth conditions which influence to transform secondary metabolites in plant. In this study, it is speculated that biosynthesis path of artemisinin (sesquiterpene) and essential oil (monoterpene) provides information of relationship with agro morphological characters for improvement of agronomic practices and will be helpful to identify superior genotypes.

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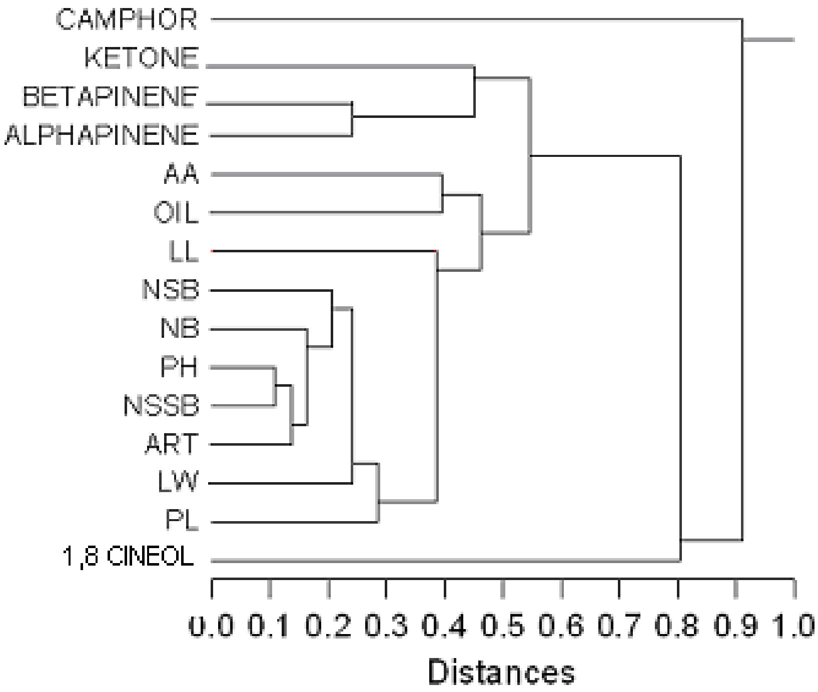

Fig 1: Relationship tree of morphological and chemical traits in *Artemisia annua*

Table 1. Correlation coefficient among morphological and chemical (monoterpenes and sesquiterpenes) of *Artemisia annua*

Characters	PH	PB	SB	TB	LL	LW	PL	Oil	Art	AA	α-pinene %	α-pinene %	α-pinene %	α-pinene %
PB	0.605**		.	.	.	.	.	.	.	.	.	.	.	.
SB	0.422**	0.438**		.	.	.	.	.	.	.	.	.	.	.
TB	0.788**	0.755**	0.471**		.	.	.	.	.	.	.	.	.	.
LL	-0.084	0.098	0.028	0.021		.	.	.	.	.	.	.	.	.
LW	0.402**	0.419**	0.313**	0.511**	0.432**		.	.	.	.	.	.	.	.
PL	0.258**	0.128*	0.202**	0.198**	0.112	0.366**		.	.	.	.	.	.	.
Oil	0.026	0.126*	0.13*	0.113	-0.055	-0.03	-0.026		.	.	.	.	.	.
Art	0.60**	0.567**	0.474**	0.705**	0.004	0.551**	0.428**	0.123		.	.	.	.	.
AA	-0.198**	-0.093	-0.069	-0.186**	-0.033	-0.130*	0.006	0.055	-0.194**		.	.	.	.
α-pinene %	-0.207**	-0.085	-0.055	-0.151*	-0.02	-0.225**	-0.072	0.153*	-0.164*	0.022		.	.	.
β-pinene %	0.014	0.144*	0.103	0.026	-0.053	-0.111	0.176**	0.175**	0.083	0.116	0.586**		.	.
Ketone %	-0.003	0.254**	0.116	0.03	0.025	0.029	0.175**	0.192**	0.147*	0.165**	0.242**	0.388**		.
camphor %	-0.385**	-0.282**	-0.191**	-0.298**	0.014	-0.239**	-0.21**	0.024	-0.307**	0.022	0.006	-0.135*	-0.416**	
1,8 cineole %	0.478**	0.299**	0.263**	0.489**	-0.062	0.247**	0.219**	0.061	0.477**	-0.091	0.011	0.147*	0.012	-0.228**

Note: PH- Plant height (cm), PB- Primary branches, SB-Secondary branches, TB- tertiary branches, LL – Lamina length (cm), LW- Lamina width (cm), PL- Petiole length (cm), Oil- Essential oil, Art- Artemisinin, AA- Artemisinic acid.
** Significant at 0.01 level, * significant at 0.05 level

Table 2. Direct (in bold) and indirect effect of different plant traits on artemisinin yield in *Artemisia annua*

Characters	PH	PB	SB	TB	LL	LW	PL	Oil	Art	AA	α-pinene	β-pinene	Ketone	Camphor	1,8 Cineole
PH	0.837	0.886	0.701	1.085	-0.022	0.881	0.689	0.003	1.184	-0.431	-0.38	0.264	0.388	-1.423	0.881
PB	0.933	0.746	0.849	1.3	0.127	1.085	0.737	0.112	1.518	-0.454	-0.248	0.849	1.536	-1.114	0.724
SB	0.891	0.729	0.629	1.827	-0.001	1.452	1.137	0.184	1.012	-0.765	-0.427	1.019	1.503	-1.704	0.721
TB	0.883	0.711	0.512	0.919	0.054	0.921	0.609	0.045	1.212	-0.555	-0.458	0.201	0.371	-1.56	0.705
LL	-0.021	0.071	0	0.052	0.037	2.1	4.482	-3.418	-0.342	-0.482	-1.688	-1.594	1.124	1.158	0.07
LW	0.672	0.485	0.393	0.701	0.32	0.725	1.01	-0.165	1.59	-0.906	-2.013	-1.571	-0.198	-1.533	0.48
PL	0.601	0.36	0.35	0.5	0.105	0.524	0.571	-0.235	2.02	-0.217	-0.222	1.472	2.051	-2.862	0.352
Oil	0.801	0.078	0.078	0.052	-0.038	-0.1	-0.093	-0.95	1.365	-1.187	3.619	4.685	8.195	-1.189	0.077
Art	0.004	0.602	0.485	0.798	-0.002	0.542	0.429	0.028	0.187	-0.537	-0.447	0.36	0.702	-1.566	0.481
AA	-0.241	-0.149	-0.128	-0.269	-0.004	-0.153	-0.039	-0.038	-0.28	-0.212	1.095	2.342	1.122	1.867	-0.124
α-pinene	-0.22	-0.082	-0.072	-0.222	-0.014	-0.346	-0.044	0.11	-0.227	0.059	-0.174	1.722	1.176	-0.355	-0.07
β-pinene	0.141	0.256	0.158	0.09	-0.04	-0.156	0.255	0.139	0.159	0.128	0.55	0.137	1.672	-0.869	0.155
Ketone	0.137	0.304	0.153	0.11	0.022	-0.008	0.217	0.121	0.206	0.129	0.178	0.347	0.217	-0.245	0.15
Camphor	-0.703	-0.473	-0.37	-0.641	0.062	-0.221	-0.349	-0.03	-0.638	0.092	-0.004	-0.23	-0.349	-0.354	-0.33
1,8 cineole	0.819	0.722	0.62	1.825	-0.001	1.452	1.133	0.181	1.011	-0.76	-0.422	1.001	1.5	-1.7	0.631

* Residual effect=0.334

Note: PH- Plant height, PB- Primary branches, SB-Secondary branches, TB- tertiary branches, LL – Lamina length, LW- Lamina width, PL- Petiole length, Oil-Essential oil, Art- Artemisinin, AA- Artemisinic acid.