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Genetic divergence in Ashwagandha [*Withania somnifera* (L.) Dunal.]: A review

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Abstract
Ashwagandha [*Withania somnifera* (L.) Dunal] is an important medicinal plant which is a major source of alkaloids, *viz.*, withanolides, sominiferine, sominiferinine, somnine, withananine, withaferine and withasomnine. Wide range of variation was observed for most of the traits in this crop. In Ashwagandha, high heritability along with high genetic advance was observed for most of the traits due to additive gene effects and consequently the scope is more for improving traits through selection. The correlations existed between the plant growth, root yield and quality components were observed revealed that a strong association was exhibited between different traits. The highest direct and positive on dry root yield per plant was observed with its yield contributing traits. D^2 analysis showed that, there was considerable divergence among the various genotypes. The clustering pattern revealed that genotypes of same geographical area fall into different clusters. This indicates that clustering pattern was influenced by genotypic constitution rather than geographical origin.

Keywords: Ashwagandha, Genetic diversity, Variability, Heritability, Genetic advance, Correlation, Path analysis, D^2 analysis

1. Introduction
India has a rich heritage of natural biodiversity of flora and fauna including medicinal plants for various purposes. Several medicinal plants are naturally grown in Western Ghats and few are being grown commercially as per its demand and commercial value. Ashwagandha [*Withania somnifera* (L) Daunal] belongs to the family *Solanceae* and is a cross pollinated crop with chromosome number 2n=48 (Nigam and Kandalkar, 1995) [29]. It is also known as Indian ginseng or *winter chery* (Deshpande, 2005) [10]. It is one of the most important medicinal plant species known to ancient Indians for centuries. It is a hardy and drought tolerant perennial plant. It is a perennial shrub and grows naturally under subtropical dry climate in well drained, sandy loam or light red soils having pH of 7.5 to 8.0 with an average rain fall of 600-750 mm. It is native of North-western and Central India as well as Mediterranean region of North Africa. Two species of Ashwagandha, *viz., W. somnifera* (L.) Dunal and *W. coagulans* (L.) are found in India.
The medicinal utility of roots is due to presence of number of alkaloids. The total alkaloid content in the roots varied from 0.16 to 0.66 per cent [Biennial Progress Report (2006-2008)]. The main alkaloids are *withanolides, sominiferine, sominiferinine, somnine, withananine, pseudo withanolides, withananinine* and with *asomnine* [Covello and Ciampa (1960)] [8]. The roots are used for general and sexual weakness in the male, female disorder, leucorrhea and neuromuscular system, prevent old age symptoms, remove acidity and prevent osteoarthritis etc (Jadhav, 2003) [17]. There are at least 5 distinct morphological forms of *Withania somnifera* and there is an extreme degree of variability with respect to growth habit and morphological characteristics of plants found in different parts of India and elsewhere [Atel and Schwarting (1962)] [2].
The Ashwagandha crop had not been domesticated into regular agriculture until recent past, but considering the important medicinal value of this crop recently a few farmers have started its commercial cultivation. Ashwagandha crop can be cultivated throughout the drier parts and in the subtropical and semi temperate regions extending from 23^0 N to 33^0 N latitude and from 180 - 1700 m altitude above sea level, including the States of Maharashtra, Madhya Pradesh, Gujarat, Rajasthan, Uttar Pradesh, Haryana, Punjab, Orisa, Sikim and Assam [Billore, 1989; Chaudhari and Vacharajani, 1992; Pandey and Dixit, 1980] [4, 7, 31].
Despite of many medicinal uses of Ashwagandha, there is a lack of improved varieties

(Jain *et al.,* 2007) [18]. Very little work has been done in the varietal characterization (Senthil, 2010) [37]. Hence identification of desirable genotypes for particular character is necessary. There is wide variability in yield and size of roots in Ashwagandha (Das *et al.,* 2011) [9]. This suggests that there is a good scope for increasing production by developing high dry root yielding varieties. Therefore, it is necessary to study the available variability and diversity in genotypes of Ashwagandha. Considering the status and low productivity of the crop, there is need to develop and identify superior genotypes by exploiting the available variation simultaneously understanding the association between various yields contributing character with their degree of divergence.

Genetic Variability

The possibility of achieving improvement in any crop plants primarily depends on the magnitude of genetic variability. Though heritability value of a trait indicates the effectiveness of selection based on phenotypic expression, though selection is the product of its heritability, phenotypic standard deviation and selection differential (Burton and Devane, 1953) [5].

Earlier efforts by Johannsen (1909) [19], Nilson-Ehle (1909) [30] and East (1916) [13] had led to the partitioning of total variability into genetic and environmental components. The classical experiment of Johannsen (1909) [19] demonstrated that both heritable and non-heritable agencies contributed to somatic variation in segregating populations and that variation in pure line was entirely due to environment. Nilson-Ehle (1909) [30] and East (1916) [13] further confirmed the work of Johannsen (1909) [19] and demonstrated how such results obtained based on the study on non-segregating populations. Charles and Smith (1939) [6], Powers (1942) [34] and Powers *et al*. (1950) [35] separated genetic variance from total variance.

The brief reviews pertaining to the genetic variability, heritability and genetic advance is summarized as under.

A clear understanding of the extent of variability prevailing for each trait in germplasm is essential for the improvement of character through selection. In hybridization programme, selection of genetically diverse parent is important to get wide range of recombinants. Based on variability analysed, Misra *et al.* (1998a) [25], Misra *et al.* (1998b) [26], Kandalkar *et al.* (1993) [22], Laximinarayan and Mukund (2003) [24], Arunkumar *et al.* (2007) [1], Iqbal and Datta (2007) [16, 28], Jain *et al.* (2007) [18], Mohsina *et al.* (2007) [28], Yadav *et al.* (2008) [41], Dubey *et al*. (2010) [12], Gupta *et al.* (2011) [15], Das *et al*. (2011) [9], Pawar (2012) [32], Mishra (2014) [27], Singh *et al* (2014) [38], Sukh Dev *et al.* (2015) [39] and Gami *et al.* (2016) [14] reported sufficient amount of variability in ashwagandha, it could be stated that there existed ample scope of variation in these characters that could be utilized for improvement through selection for the characters investigated. The estimates of PCV were slightly higher than those of GCV indicated the extent of the environment influence on traits. Large differences between PCV and GCV values effect high environmental influence on the expression of traits while slight difference indicated minimum environmental influence and consequently greater role of genetic factor on the expression of traits. The higher phenotypic and genotypic coefficients of variation were recorded for dry root yield by Misra *et al.* (1998) [25, 26], Arunkumar *et al.* (2007) [1], Kumar *et al.* (2008) [41], Yadav *et al.* (2008) [41], Dubey (2010) [12], Sundesha and Tank (2013) [40], Mishra (2014) [27], Joshi *et al.* (2014) [20] and Sukh Dev *et al.* (2015) [39], while moderate phenotypic and genotypic coefficients of variation were recorded for dry root yield by Singh *et al.* (2014) [38]. The higher phenotypic and lower genotypic coefficients of variation were recorded for fresh root yield by Pawar (2012) [32], Sangwan *et al.* (2013) [36], Mishra (2014) [27] and Singh (2014) [38]. The higher phenotypic and genotypic coefficients of variation were recorded for seed yield per plant by Yadav *et al.* (2008) [41], Pawar (2012) [32] and Sangwan *et al.* (2013) [36] while moderate genotypic and higher phenotypic coefficients of variation were recorded by Singh *et al.* (2014) [38] and lower phenotypic and lower genotypic coefficients of variation were recorded by Sundesha and Tank (2013) [40] and Sangwan *et al.* (2013) [36]. Early flowering is generally an indication of early yield and also early varieties fit well in multiple cropping systems. For days to 50% flowering, Pawar (2012) [32], Sangwan *et al* (2013) [36], Sundesha and Tank (2014) [40], Singh *et al.* (2014) [38] and Sukh Dev (2015) [39], exhibited lower phenotypic and genotypic coefficients of variation in ashwagandha. Misra *et al.* (1998) [25, 26] recorded higher phenotypic and genotypic coefficients of variation for plant canopy. The ideal plant type should have longer plant height and number of branches was the major parameter which acts as source trait to support yield and its component traits. For plant height, higher phenotypic and genotypic coefficients of variation was recorded by Arunkumar *et al.* (2007) [1], Yadav *et al.* (2008) [41], Mishra (2014) [27] and Sukh Dev *et al.* (2015) [39] and lower phenotypic and genotypic coefficients of variation were recorded by Misra *et al.* (1998) [25, 26], Sundesha and Tank (2013) [40], Sangwan *et al.* (2013) [36] and Singh *et al.* (2014) [38] while moderate phenotypic and genotypic coefficients of variation was recorded by Pawar (2012) [32] and Joshi *et al.* (2014) [20]. Number of branches per plant is one of the major parameter contributing for total fruit yield per plant. The higher genotypic and phenotypic coefficient of variation was recorded number of branches per plant by Arunkumar *et al.* (2007) [1], Pawar (2013) [32], Sundesha and Tank (2013) [40], Joshi *et al.* (2014) [20] and Sukh Dev *et al.* (2015) [39], while moderate was recorded by Singh *et al.* (2014) [38]. The higher phenotypic and genotypic coefficients of variation for Withanoloide content (%) was recorded by Sundesha and Tank (2013) [40], Joshi *et al.* (2014) [20] and Mishra (2014) [27] and Sukh Dev *et al.* (2015) [39], while the lower was recorded by Sangwan *et al.* (2013) [36] and Mishra (2014) [27]. For number of seeds per plant, higher phenotypic and genotypic coefficients of variation was observed by Yadav *et al.* (2008) [41] while moderate was observed by Mishra (2014) [27]. For number of fruits per plant, higher phenotypic and genotypic coefficients of variation was observed by Mishra (2014) [27]. For days to maturity, lower phenotypic and genotypic coefficients of variation was observed by Pawar (2012) [32].

Heritability and Genetic advance

Suggested that the use of heritability estimates with genotypic coefficient of variation to give precise estimate of genetic advance. Pointed out that in a selection programme heritability values as well as estimates of genetic advance are more useful than heritability alone. According to heritability and genetic advances are the two complementary concepts. The heritability values may be used to estimate the expected genetic advance through selection. The heritability enables the plant breeder to base his selection on phenotypic performance for improvement of character.

Estimation of heritability and genetic advanced were high for dry root yield per plant [Sundesha and tank (2013) [40], Joshi *et al.* (2014)] [20], seed yield per plant [Sangwan *et al.* (2013)]

[36], fresh root yield per plant [Sangwan *et al.* (2013)] [36], average diameter of root per plant [Singh *et al.* (2014)] [38], root length [Sangwan *et al.* (2013) [36], Joshi *et al.* (2014)] [20], root volume per plant [Sangwan *et al.* (2013) [36], Joshi *et al.* (2014) [20], Singh *et al.* (2014)] [38], number of capsules per plant [Sundesha and tank (2013) [40], Sangwan *et al.* (2013) [36], Singh *et al.* (2014)] [38], number of branches per plant [Sundesha and tank (2013) [40], Joshi *et al.* (2014) [20], Singh *et al.* (2014)] [38], number of seeds per berry [Sangwan *et al.* (2013)] [36], total alkaloids [Sundesha and tank (2013) [40], Joshi *et al.* (2014)] [20] and plant height [Joshi *et al.* (2014) [20], Sangwan *et al.* (2013)] [36], in genotypes indicating the predominance of additive gene action for these traits, hence direct selection may be highly effective. So these characters may be considered as important criteria for selection of the parent for hybridization program. The lower estimation of heritability and genetic advanced were observed for plant height [Sundesha and tank (2013) [40], Singh *et al.* (2014)] [38], root diameter [Sundesha and tank (2013), Singh *et al.* (2014)] [38], root length [Sundesha and tank (2013) [40], Singh *et al.* (2014)] [38], days to flowering [Sundesha and tank (2013) [40], Singh *et al.* (2014)] [38], days to maturity [Sundesha and tank (2013) [40], Joshi *et al.* (2014)] [20], dry root yield per plant [Singh *et al.* (2014)] [38] and fresh root yield per plant [Singh *et al.* (2014)] [38] indicating the predominance of non-additive gene action for these traits, hence direct selection was not effective.

Correlation and Path Analysis

Correlation studies are helpful in determining the yield components, but do not provide an exact picture of the relative importance of direct and indirect influence of each of the component characters towards yield, path analysis, correlation coefficient measures of direct and indirect effects and measures the direct and indirect combination of various independent variables on the dependent variable (Dewey and Lu, 1959) [11]. The association between root yield and other characters was significantly positive in most of the characters. Plant height showed significant positive association with root length, root diameter, biological yield and root yield per plant. Root length exhibited positive and significant association with plant height, root diameter, number of berries per plant, biological yield, seed yield per plant and root yield per plant. Studies of association in Ashwagandha were also conducted by Pol *et al.* (2003), Yadav *et al.* (2008) [41], Mohshian Iqbal and Datta (2007) [16, 28], Kubsad *et al.* (2009), Kumar *et al.* (2012), Pawar (2012) [32], Sangwan *et al.* (2013) [36], Joshi *et al.* (2014) [20], Mishra (2014) [27], Sukh Dev *et al.* (2015) [39] and Gami *et al.* reported almost similar results.

In addition to the degree of association, path coefficient analysis takes into account the cause and effect relationship and has been performed to partition correlation matrix into direct and indirect effects for understanding the relative importance of the component characters on yield. In recent studies, positive direct contribution on seed yield was demonstrated by number of berries per plant, number of primary branches per plant, fresh weight of leaves and fresh weight of plant. As the roots are medicinally important in ashwagandha, direct contribution on root yield (fresh weight) was also assessed by several worker and it was emphasized that fresh weight of plant had maximum direct positive direct effect. Negative direct effect of leaf length, days to flower initiation, number of secondary branches, withanoloid and starch content on dry root yield. Residual effect was noted to be low. Similar results were also obtained by Kandalkar *et al.* (1993) [22], Mohshina Iqbal and Datta (2007) [16, 28], Yadav, *et al.* (2008) [41], Kubsad *et al.* (2009), Dubey (2010) [12], Rameshkumar *et al.* (2011) Sangwan *et al.* (2013) [36] and Joshi *et al.* (2014) [20] in ashwagandha.

Genetic Divergence by D^2

Genetic advance along with heritability helps to ascertain the possible genetic control for any particular trait. It is, therefore, necessary to classify and utilize this variability systematically for genetic upgradation of biological population. The D^2 statistics as suggested by and clustering suggested by helps to search genetically diverse types for hybridization programme. The inter-cluster distance is higher than the intra-cluster, indicating the wide genetic diversity among the genotypes. The greater the distance between clusters wider the genetic diversity between the genotype. Highly divergent genotype would produce a broad spectrum variability in subsequent generations enabling further selection and improvement. The hybrids developed from the selected genotypes within the limit of compatibility of these clusters may produce desirable trasgressive segregants of higher magnitude of heterosis [Singh *et al.* (2014) [38], and Joshi *et al.* (2015)] [21].

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