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SHORT COMMUNICATION

# Collection and Evaluation of Henna (*Lawsonia inermis* L.) Germplasm from South India

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An extensive germplasm exploration survey of *Lawsonia inermis* was undertaken to identify superior germplasm. Morphometric evaluation showed significant (P<0.001) variation for leaf morphometric traits and lawsone content. Total leaf dry weight (99.76 g), lawsone content (21.5 $mg^{-1}$ g dw) and lawsone yield/tree (2.14 g) were maximum in candidate elite plant 16, thus selected as 'elite' germplasm of *L. inermis*.

**Key Words: Elite germplasm, *Lawsonia inermis*, Lawsone content, Morphological evaluation, Variability**

*Lawsonia inermis* L. (syn: *L. alba* Lam., common name: Henna), the only species under the genus *Lawsonia* L. belonging to the family Lythraceae, is a small tree growing up to 12 m high. The colouring principle present in henna is lawsone (2-hydroxy-1,4 naphthoquinone), which occurs in quantities of 0.5-1.5% in dried leaf (Singh *et al*., 2014); its concentration varies in relation to environmental factors. Lawsone has proved as a non-toxic natural dye reported to have hepatoprotective, antioxidant and antimicrobial activities. This species is native of North Africa and South West Asia, and cultivated widely for the commercial production of henna leaf powder. The recent market trend indicates that the demand of henna leaf powder is increasing worldwide, thus people in many parts of India ventured commercial cultivation of *L. inermis*. However, in Kerala, the southernmost state of India, though the plant is naturalized and distributed throughout, organized cultivation is not yet initiated. It is mainly due to the poor market system coupled with the availability of comparatively low-priced products emerging from northern India. Market potential of henna largely depends on lawsone content of the leaf powder. Thus efforts to screen the germplasm having high content of lawsone coupled with other quality attributes deserve due attention. In this direction, selection of candidate elite plants (CEPs) and their morphological characterization, estimation of lawsone and analyzing correlation between growth characters will be a useful approach.

To achieve this goal, an extensive germplasm exploration survey of *L. inermis* was undertaken in different locations of Kerala and Tamilnadu states of India during 2012. To compare various yield characteristics, at first, 108 plants were randomly sampled in the study area with two prime quantitative parameters, viz., number of fresh leaves weighing 1 g and lawsone content (Dhiman *et al*., 2012). Mean value of these parameters in 108 samples was determined. Among 108 samples, 20 plants which showed 50% less number of leaf weighing 1 g than mean value and 50% higher lawsone content than mean of 108 plants were further selected for the detailed examination through single location selection programmes. These 20 plants were designated as CEPs and were subjected to detailed yield evaluation based on various quantitative traits. Collection details such as locality, latitude and longitude of 20 CEPs are as follows; CEP1 (Kariavattom, 8º33.539"N; 76º53.113"E), CEP2 (Kariavattom, 8º33.534"N; 76º55.106"E), CEP3 (Palarivattom, 10º00.128"N; 76º18.807"E), CEP4 (Poovaloor, 10º01.926"N; 76º42.407"E), CEP5 (Ayakkad, 10º36.208"N; 76º28.487"E), CEP6 (Kaapistore, 10º13.272"N; 77º08.132"E), CEP7 (Paisa Nagar, 10º14.388"N; 77º11.041"E), CEP8 (Kambiline, 10º02.255"N; 77º00.768"E), CEP9 (Kaithakkal, 11º45.600"N; 76º04.81"E), CEP10 (Devagiri 11º28.647"N; 76º21.184"E), CEP11

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(Kaithkuzhi, 11º28.205”N; 76º23.520”E), CEP12 (Devala, 11º28.526”N; 76º22.478”E), CEP13 (Korachal, 11º31.297”N;.106 ”E), CEP 14 (Kayyunni, 11º32.660”N; 76º15.660”E), CEP15 (Parashuramapuram, 10º09.820”N; 77˚43.301”E), CEP16 (Parashuramapuram, 10º09.820”N; 77º43.301”E), CEP17 (Parashuramapuram, 10º09.820”N; 77º43.301”E) CEP18 (Kovilkadavu, 10º15.224”N; 77º10.083 ”E), CEP19 (Sattur, 9º21.457”N; 77º54.939”E), CEP20 (Kalthmmuri, 9º28.738N; 76º20.192”E).

To compare yield of 20 CEPs at a single location, the branch cuttings (approx. 40 cm) were collected from these plants during monsoon season (Mid May-August 2012) and were planted in experimental plot at 1.5 × 1.5 m spacing in the Department Garden, Department of Botany, University of Kerala, Kariavattom (8º34’02.77”N; 76º53’13.42”E; altitude 42 m asl). Area receives an annual rainfall of 1500 mm, mainly through southwest monsoon during June to September. Mean monthly temperature varies between 22ºC in January and 33ºC in April. For each CEP, six cuttings were successfully established in the garden. After three years of planting, metric morphological traits of each CEP were determined and were continued for the next two successive years. Mature leaves from each CEP were collected and labeled properly. The leaves were shade-dried for one week and powdered (5 g) followed by subjected to soxhlet extraction (48 h; 37ºC) using 40 ml methanol (AR Grade, SISCO, Mumbai, India) as solvent. Quantitative estimation of lawsone (Dhiman *et al*., 2012) in air dried extract was carried out by using UV Vis spectrophotometer (Shimadzu, Japan, Model Name: UV-1700 Pharma Spec, Model No. 1700) at 452 nm, based on lawsone standard (Sigma Chemical Co., St. Louis, USA). Lawsone quantification was repeated three times for all the randomly sampled trees and 20 CEPs. For yield traits, ten replications per trait per plant were carried out. CEPs established in the experimental garden were scored during hot summer season (March-April). Scored data were subjected to one-way analysis of variance (ANOVA) and mean separation was performed using Duncan’s New Multiple Range Test ($P<0.05$). Spearman rank correlation coefficients were performed to determine the relationship between leaf yield traits as well as lawsone content (SPSS package version 22, IBM Corp. Released 2013, IBM SPSS Statistics for Windows, Armonk, NY, USA) to find P value of less than 0.05 as threshold for statistical significance.

Genetic improvement of a crop, by trait specific selection can be achieved through morphometric and biochemical traits. In order to meet the ultimate goal of high yield of the plant, evaluation of morphometric variability and selection of superior gemplasm will be beneficial. In the present study, total lawsone yield is considered as the prioritized goal in *L. inermis* for selection of superior germplasm for future breeding programmes. Mean number of leaves (61.8) weighing 1 g and mean lawsone content (10.32 mg $g^{-1}$ dw) were estimated among 108 collections and found that 20 plants are superior for both the evaluated traits, and were therefore compared further through one site yield trials. Metric characters revealed significant differences among 20 CEPs of *L. inermis* (Table 1). Leaf morphometric traits among CEPs such as length of leaf, width of leaf, length of petiole, leaf area showed significant ($P<0.001$) variation (Table 1). This significant variation might explain the genetic divergence of the germplasm originally collected from different agro-climatic regions of South India.

In the present study, morphological traits were used for characterization of different collections of *L. inermis*. In the previous studies also, such a wide range of variability in different accessions of *L. inermis* was reported (Singh *et al*., 2008). Number of leaves weighed 1 g was least in CEP6 where 15.3 freshly collected leaves constituted 1 g, substantiating large-sized leaves in this collection (Table 1). Maximum number of leaves (30.4 nos.) was required to weigh 1 g in CEP 18, characterized by narrow long leaves (Table 1). Total leaf dry weight data of 20 CEPs scored during three successive years showed highest dry weight in CEP16 (99.76 g dw). As per morphometric traits recorded, CEP6 showed some desired traits such as highest leaf length (5.38 cm) and leaf area (11.36 $cm^2$). However, superiority in leaf length and area estimates is not enough to elevate a CEP to elite category. The spectrophotometric determination of lawsone content in 20 collections of *L. inermis* showed significant ($P<0.001$) variation among collections which was planted in same climatic conditions (Table 1). The lawsone content in selected CEPs varied from 15.4 to 23.1 $mg^{-1}$ g dw. Highest amount of lawsone was estimated in CEP15 (23.1 $mg^{-1}$ g dw). The CEP6 recorded least (15.4 $mg^{-1}$ g dw) lawsone content. Our findings on lawsone content of *L. inermis* suggest that a comparable range of lawsone is present in most of the CEPs studied with that of northern arid zone accessions

**Table 1. Leaf morphometric traits of different candidate elite plants (CEPs) of *L. inermis* planted in the experimental plot.**

| Collection Code | Leaf length (cm) | Leaf width (cm) | Petiole length (cm) | Leaf area ($cm^2$) | Total no. of main branches/ plant | Mean no. of leaves /sub-branch | No. of leaf weighing 1 g | Total leaf dry weight (g) | Lawsone content ($mg^{-1}$ g dw) | Total lawsone yield/tree (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| CEP1 | 3.87gh | 1.51ij | 0.55e | 5.85m | 12.4d | 14.3b | 28.2c | 51.43gh | 21.2de | 1.09f |
| CEP2 | 3.84g | 1.51ij | 0.57cd | 5.83m | 14.0bc | 12.7c | 30.3a | 29.56k | 21.4d | 0.63j |
| CEP3 | 4.31f | 1.46j | 0.50f | 6.15j | 13.1c | 12.2c | 26.2c | 57.35f | 21.2de | 1.22e |
| CEP4 | 3.74hi | 1.57hi | 0.46gh | 5.97l | 14.1bc | 14.5b | 22.4d | 75.09d | 18.4g | 1.38d |
| CEP5 | 4.32d | 1.71ef | 0.47gh | 7.15g | 13.2c | 10.5d | 29.1bc | 38.56j | 17.1h | 0.66j |
| CEP6 | 5.38a | 2.11ab | 0.72b | 11.36a | 11.9d | 14.4b | 15.3e | 92.80b | 15.4j | 1.43d |
| CEP7 | 3.48j | 1.52ij | 0.45h | 5.35n | 13.3c | 12.3c | 30.1a | 43.10i | 22.8ab | 0.98g |
| CEP8 | 2.89k | 1.45j | 0.31k | 4.18o | 13.1c | 14.8b | 26.2c | 45.00i | 21.3de | 0.96g |
| CEP9 | 3.82gh | 1.61gh | 0.34j | 6.08k | 12.2d | 10.5d | 27.5c | 49.26h | 16.8i | 0.83h |
| CEP10 | 4.06f | 1.46j | 0.46gh | 5.85m | 13.8bc | 12.2c | 19.7de | 34.66j | 18.4g | 0.64j |
| CEP11 | 3.94fg | 1.78de | 0.47fgh | 7.03i | 15.0ab | 10.4d | 21.1d | 62.86e | 22.2bc | 1.39d |
| CEP12 | 4.73c | 2.15a | 0.67c | 10.05d | 12.2d | 12.7c | 21.2d | 43.42i | 20.4f | 0.89hh |
| CEP13 | 3.70i | 1.56hi | 0.72b | 5.87m | 12.4d | 10.2d | 18.1de | 49.27h | 21.2de | 1.04fg |
| CEP14 | 5.16c | 2.11ab | 0.93a | 10.57b | 13.2cd | 12.7c | 19.0d | 52.00gh | 17.3h | 0.99g |
| CEP15 | 4.12f | 2.05b | 0.41i | 8.49e | 14.1b | 14.8b | 21.2d | 86.66c | 23.1a | 2.01b |
| CEP16 | 4.37d | 1.67fg | 0.65c | 7.33f | 16.2a | 14.3b | 17.0e | 99.76a | 21.5cd | 2.14a |
| CEP17 | 3.82gh | 1.86c | 0.48fg | 7.13g | 15.1a | 10.2d | 22.2d | 53.18fg | 18.5g | 0.98g |
| CEP18 | 3.83g | 1.85cd | 0.47fgh | 7.06h | 14.9a | 12.4c | 30.4a | 34.80j | 20.6ef | 0.72i |
| CEP19 | 3.72i | 1.68fg | 0.48fg | 6.26i | 16.1a | 16.2a | 19.3de | 87.57c | 20.7def | 1.81c |
| CEP20 | 4.72c | 2.17a | 0.59d | 10.22c | 12.8c | 10.3d | 22.2d | 46.09i | 16.2i | 0.75i |
| F value DF (n-1)= 19 | 453*** | 101*** | 339*** | 783.8*** | 114*** | 87.0*** | 76.3*** | 184.7*** | 90.8*** | 46.5*** |

***Significant at P<0.001 level; Means within column followed by same letter are not significantly (P<0.05) different as determined by Duncan's New Multiple Range test (DNMRT).

(Singh *et al*., 2008), where mean content among selected accessions was 19.8 $mg^{-1}$ g dw (1.98%). In the present study, mean lawsone content of 108 randomly sampled individuals was 10.32 $mg^{-1}$ g dw and best selected one (CEP16) had 21.5 $mg^{-1}$ g dw lawsone content, which is 100% higher than the mean. Present finding is in agreement with previous report that leaf size and lawsone contents varied significantly among sampled accessions of *L. inermis* (Singh *et al*., 2008). Possibly, differences in regulation of synthesis of phytochemicals due to varying activity of regulating enzymes explained genetic differences of the plant.

Spearman rank correlation coefficients among the ten quantitative traits showed significant inter correlation of four traits with lawsone yield. Yield characters such as mean number of leaf/sub-branch (r=0.610), no. of leaf weighing 1 g (r=0.488) and total leaf biomass of plant (r=0.940) showed significant positive correlation with total lawsone yield of the plant. The leaf length was highly positively correlated with the leaf width (r=0.716) and leaf area (r=0.926), and negatively correlated with lawsone content (r=-0.574). Correlated quantitative traits are of major interest in crop improvement programme, as the improvement of one character may cause simultaneous changes in other characters. As long as the genes governing the characters are not combined at random, characters may show some correlation. If the observed correlation is due to multiple effects of a same gene, the selection for one character will affect other characters. Hence, correlation among the traits influences effectiveness of selection. Among various morphometric traits and lawsone yield, the lawsone yield was considered as the most prioritized trait which showed strong relationship with biomass and lawsone content. The tree having highest lawsone yield, which is a direct product of leaf biomass and lawsone content, was screened as "elite". Yield characters such as mean number of leaf/sub-branch (r=0.610) and total biomass of leaf per plant (r=0.79) showed significant positive correlation with total lawsone yield of the plant. Based on highest leaf dry biomass and maximum lawsone yield (2.14 g), CEP16 was selected as elite or plus tree. The selected CEP showed 100% higher lawsone content (21.5 $mg^{-1}$ g dw) than mean content of 108 randomly sampled individuals. Thus best selected accession CEP16 can further be used for the clonal propagation both through

conventional and biotechnological tools and in breeding programmes.

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