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Selecting High Forage-yielding Alfalfa Populations in a Mediterranean Drought-prone Environment by Using High-throughput Phenotyping

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Abstract : Introduction: One of the primary environmental factors affecting forage crop yield globally is drought, particularly in Mediterranean climatic conditions, where drought typically persists for 5-6 months, usually between October and March in countries like Chile. Alfalfa, a perennial forage crop with deep roots, employs a diverse range of drought-tolerant strategies at the physiological, morphological, and molecular levels. In the current study, 250 alfalfa half-sib populations containing different genetic makeups were grown for three growing seasons (2021 to 2023) to identify drought-resistant populations with high forage yield in two water regimes (irrigated and rainfed) under the Mediterranean drought-prone region of Central Chile, Cauquenes. The objectives were to i) develop new field phenotyping methods using remote sensing technologies such as Red-Green-Blue (RGB) and thermal cameras to identify high-yielding and drought-tolerant alfalfa populations, and ii) select outstanding genetic material for plant breeding. Material And Methods: Field phenotyping involves using remote sensing technology, including RGB and thermal cameras mounted on unmanned aerial vehicles, and measuring the forage yield of 250 alfalfa half-sib populations grown under rainfed and irrigated water regimes in a Mediterranean drought-prone environment, during three growing seasons (2021-2023). Both trials were arranged in an α-lattice experimental design with two replications. Each replicate has 10 partial blocks including 25 half-sib populations. RGB-derived indices and canopy temperature difference (CTD), determined by subtracting the canopy temperature (Tc) from the ambient temperature (Ta), were related with forage yield. Results And Discussion: Results indicate that forage yield exhibited significant variability among the alfalfa populations, in both rainfed and irrigated conditions. During winter, it ranged from 1.4- to 6.1 Mg ha-1 in rainfed conditions and from 1.4 to 8.2 Mg ha-1 under the irrigated regime. Total forage yield ranged from 3.7 to 14.7 Mg ha-1 in rainfed conditions and from 6.3 to 25.1 Mg ha-1 in the irrigated regime. Among half-sib populations, the most productive populations were AlfaL4-5 (parent SARDI7), AlfaL57-7 (parent WL903), and AlfaL62-9 (parent Baldrich350), which produced the highest (>13 Mg ha-1 mean total FY and > 4.5 Mg ha-1 mean winter FY during 2021-2023) forage yield in both water regimes. RGB indices Hue, Saturation, b*, v*, GA, and GGA exhibited positive correlations, whereas Intensity, Lightness, a*, and u* showed negative correlations with forage yield in both water regimes. In 2021, RGB-derived indices showed a weak correlation ($r < 0.5$) with CTD. However, strong correlations were observed in November 2022 ($r = -0.8$ to $+0.8$) and 2023 ($r = -0.7$ to $+0.7$), specifically in the irrigated regime, indicating better performance under higher water availability. Moreover, the CTD was negatively correlated with FY ($r = -0.28$ for rainfed and -0.32 for irrigated in 2021, $r = -0.57$ for rainfed and $r = -0.76$ for irrigated in 2022, and $r = -0.34$ for rainfed and $r = -0.52$ for irrigated in 2023) of 250 alfalfa half-sib populations. It is concluded that CTD and RGB-derived indices were the most effective tools for identifying drought-resistant populations grown in Mediterranean drought-prone environments. In rainfed alfalfa, the most highly productive populations were AlfaL29-4 (parent AS3), AlfaL61-9 (parent Genesis), and AlfaL4-7 (parent SARDI7). Meanwhile, in irrigated conditions, the alfalfa half-sib populations AlfaL56-4 (parent Venus) and AlfaL57-2 (parent WL903) demonstrated maximum FY. Conclusion: Alfalfa winter and total FY varied widely between the three growing seasons (2021-2023) under two water regimes, rainfed and irrigated. There were three alfalfa half-sib populations, AlfaL4-5 (parent SARDI7), AlfaL57-7 (parent WL903) and AlfaL62-9 (parent Baldrich350), that exhibited high FY in both water regimes, rainfed and irrigated. The thermal camera derived index CTD (Tc-Ta) showed negative correlation with FY and appeared to be the most powerful tool in identification of alfalfa genotypes grown under Chilean Mediterranean drought prone environment.

Keywords : alfalfa, remote sensing, phenotyping, forage crop
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