

Mescaline distribution and concentration in *Trichocereus bridgesii*

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Abstract

Trichocereus bridgesii Britton & Rose is a widely cultivated botanical source of mescaline with a long history of use as a San Pedro cactus. Despite its reputation as a mescaline-rich species, quantitative data remain limited, and mescaline distribution within individual plants is poorly understood. This study characterised variation in mescaline concentration among cultivated *T. bridgesii* specimens, tissues and spatial positions using complementary sampling approaches and a validated UHPLC–ESI–MS/MS method. Mescaline concentrations varied substantially among cultivar-labelled specimens and within individual plants. Chlorenchyma generally contained significantly higher concentrations than paired parenchyma, although exceptions occurred. Transverse grid dissections demonstrated higher concentrations towards the stem periphery with pronounced fine-scale heterogeneity. Vertical profiling revealed substantial heterogeneity along stems and between adjacent stems, without evidence of a consistent apical-to-basal gradient. In contrast, concentrations were comparatively consistent around the circumference of the two plants examined. Substantial variation was also observed among replicated samples of ‘TBM Clone B’ and across a 64-specimen cultivar screening panel. Mescaline distribution in cultivated *T. bridgesii* was therefore heterogeneous across multiple biological scales. Measurements from a single tissue or sampling position may not reliably represent an entire stem or plant, highlighting the importance of representative sampling in phytochemical analyses.

1. Introduction

San Pedro, an informal group of cacti comprising approximately 20 closely related mescaline-containing species, have long been used in Andean ceremonial traditions and are now attracting renewed scientific and clinical interest. *Trichocereus bridgesii* Britton & Rose is among the mescaline-containing Andean cacti reported to be used by Indigenous practitioners for psychoactive diagnostic and therapeutic purposes [1]. Cacti within the San Pedro group are often classified as *Trichocereus* [2], however more recent taxonomic treatments have variously included these species within *Echinopsis* [3] or *Leucostele* [4]. The name *Trichocereus* is retained throughout this manuscript for consistency with the ethnopharmacological and phytochemical literature. Archaeological sites at Las Aldas on the north-central Peruvian coast may be evidence of early – circa 2000 – 1500 BCE – ritual San Pedro use [5]. Regionally continuous ceremonial traditions incorporating both Indigenous ritual frameworks and colonial-era Catholic symbolism persist in parts of the Andes where these cacti occur naturally [6]. From a reductionist perspective, the pharmacological activity of San Pedro is principally attributed to a substituted pentylamine compound known as mescaline (syn. mezcaline); an agonist of the 5-hydroxytryptamine type 2A (5-HT_{2A}) receptor that can alter consciousness and perceptions of reality [7]. However, from a holistic perspective, the structure of and participation in a ritual practice may be important modulators of psychological effects. Mescaline administered in screened, controlled clinical research, including as a potential adjunct to psychotherapy, may have a substantially different benefit–risk profile from self-administration, although direct comparative evidence and controlled evidence of mescaline’s psychotherapeutic efficacy remain limited [8–10].

Outside their native range, San Pedro are highly amenable to widespread cultivation owing to their rapid growth and resilience in temperate climates. As wild populations of Peyote (*Lophophora williamsii* (Lem. ex Salm-Dyck) J.M.Coult.) have become increasingly threatened, cultivation of San Pedro has assumed growing conservation importance as an alternative source of mescaline-containing cacti and has been promoted as a means of reducing harvesting pressure on Peyote [11–13]. Whilst increasing international demand has raised concerns about unsustainable harvesting of some native *Trichocereus* populations, current evidence suggests these impacts are geographically localised and often compounded by broader habitat loss rather than representing a demonstrated species-level conservation threat (Engel & Ostolaza, pers. comms. 2023) [14].

Despite the long history of San Pedro and a growing clinical interest in psychedelic compounds, little is known about the distribution of mescaline within individual plants. Published mescaline concentrations in the San Pedro species vary widely, but it remains uncertain whether this variation primarily reflects biological differences between species and cultivars, methodological differences in tissue selection and analysis, or an interaction between these factors. Most studies of *T. bridgesii* have examined small numbers of plants or cultivars and frequently analysed only outer cortical tissue (Table S1). More recently, Gaur et al. [15] demonstrated substantial differences in mescaline concentration between chlorenchyma and parenchyma in three cultivars, indicating that tissue selection can materially affect measured concentration. However, mescaline distribution within individual stems has not been systematically characterised. Understanding this distribution and ensuring representative phytochemical sampling has implications relevant not only to ethnopharmacological research but also to cultivation, harm reduction and interpretation of historical and contemporary preparation methods.

Accordingly, the present study investigated variation in mescaline distribution within cultivated *T. bridgesii* across radial, vertical and circumferential positions of individual stems. This investigation informed a representative sampling technique that was applied to study the difference in mescaline concentration between chlorenchyma and parenchyma. Within-cultivar variation was assessed using 22 plantings of the distinct and widely cultivated clone ‘TBM Clone B’. Variation across the cultivar screening panel was assessed using 64 cultivar-labelled *T. bridgesii* specimens.

2. Materials & Methods

2.1 Sampling scheme

Plant material was obtained from cultivated *T. bridgesii* plants maintained in Australian horticultural collections, including The Mescaline Garden and collaborating specialist collectors. Species identifications were based on morphology and horticultural provenance; genetic authentication and comparison with herbarium voucher specimens were not undertaken. Named cultivars and clones were identified according to the provenance and names under which they are maintained in specialist collections and are therefore treated as horticultural identifiers rather than genetically verified taxonomic units. Cultivars were selected based on availability, with the aim of maximising the diversity of cultivated

forms examined. Most cultivars in this screening were represented by a single plant; consequently, the analysis characterises variation among cultivar-labelled specimens rather than estimating replicated cultivar means. *T. bridgesii* 'TBM Clone B' is an internationally distributed horticultural clone maintained through vegetative propagation and widely cultivated in collections worldwide. For the 'TBM Clone B' investigation, samples were obtained from multiple gardens and independent plantings to capture variation within this widely cultivated cultivar. Grid, vertical and circumferential sampling was undertaken using plants of suitable size and morphology for destructive sampling.

Where plants were of sufficient size, samples were collected as small cuttings or wedges. Where sacrificial sampling of a stem or rib was not feasible or permitted by the donor, tissue was collected in a minimally destructive way by using a 4-gauge biopsy needle punched perpendicularly into the core from the junctions between two ribs. Fresh cortex was separated into chlorenchyma and parenchyma, with areoles and spines removed before processing.

Initial investigations of distribution within specimens included: (i) 6 × 6 grid-based cross-section, taken by dissecting a transverse section (~ 30 mm) of two *T. bridgesii* cultivars ('Experiment' and 'TCF1'), (ii) vertical profiling, by taking ten equidistant sampling points along mature stems and additional matched apical-basal samples from additional stems, utilising two cultivars ('Experiment' and 'Nimbin') cultivated at the same location, and matched apical-basal samples only for three individuals of a third cultivar ('TBM Clone B'); and (iii) circumferential profiling, by taking transverse rib segments from cuttings of two cultivars ('Kai' and 'Nimbin'). The distribution studies informed a standardised sampling strategy in which tissue is collected from mature stems at least 150 mm from either the apex or the base of the stem (cuttings of > 150 mm are a typical length for propagating clones). Samples may comprise a complete transverse section, a single rib, or a biopsy taken from an arbitrary position around the circumference. The standardised sampling was applied to: (iv) screening of parenchyma and chlorenchyma from multiple stems and plantings (25 stems from 22 plantings) of the same cultivar ('TBM Clone B'); and (v) screening paired parenchyma and chlorenchyma samples from 64 cultivar-labelled *T. bridgesii* specimens.

2.2 Sample preparation and quantitative analysis of mescaline

Samples were refrigerated following collection and freeze-dried to constant mass, typically within 48 h of harvesting, using a Christ Alpha 1–4 LD plus freeze dryer (John Morris Scientific; Chatswood, NSW, Australia). Wedge and cutting samples were homogenised to a fine powder using a NutriBullet NB9-1107AK blender (Los Angeles, CA, USA). Duplicate independent sample preparations were performed for homogenised wedge and cutting samples by preparing two separately extracted aliquots from the same homogenised biological sample. Needle biopsy samples were processed without homogenisation owing to the limited amount of material available and were therefore analysed from a single soaked preparation.

All samples were extracted with methanol at a ratio of 1 mL solvent per 1 mg of dried biomass. Extraction was aided using 30 min low-power sonication in a Powersonic 420 ultrasonic bath (Thermoline Scientific; Sydney, NSW, Australia). Needle biopsied samples required a further passive soak overnight (not less than 18 hours), and an additional 30 min sonication the following day. Sample solutions were filtered through 0.22µm Nylon before UHPLC–ESI–MS/MS analysis of mescaline according to a previously validated method [15].

2.3 Validation of sampling by minimally destructive needle biopsy

Initial validation of the minimally destructive needle biopsy sampling technique was undertaken by comparison with the previously validated sample homogenisation and preparation scale. A *Trichocereus pachanoi* Britton & Rose specimen with six ribs was selected as the control specimen, as tissue distribution has previously been characterised in this species [16, 17], allowing comparison with an established anatomical framework. The specimen had a nominal chlorenchyma mescaline concentration of 10 mg/g. A 30 mm transverse section was collected and the ribs were separated. All parenchyma was discarded, and analysis was performed only on the chlorenchyma. Each rib was sampled once using a biopsy needle, which was dried and extracted whole, whilst the remaining material was prepared using the conventional approach of drying, homogenisation and subsampling [15].

2.4 Descriptive and statistical analysis

Mescaline concentrations are reported on a dry weight basis, unless stated otherwise. Duplicate preparations from the same homogenised biological sample were reported as the mean $\pm$ standard deviation (SD) of the sample preparations; they were not treated as independent biological replicates for subsequent statistical analyses. Duplicate sample preparations were performed to assess variability introduced during sample preparation rather than biological variation. Alternatively, concentrations in needle biopsy samples were reported as the mean $\pm$ standard deviation (SD) of duplicate instrumental analyses, occasionally with negligible variances. Descriptive statistics for biological samples include mean $\pm$ standard deviation (SD), median, interquartile range and range.

Statistical analyses were performed in R (version 4.6.1) with R Commander (version 2.13.0) as a GUI. Comparisons of the matched apical and basal needle biopsies were made by the binomial sign test. Paired chlorenchyma and parenchyma concentrations were compared using Wilcoxon signed-rank tests, whilst associations between the paired tissue concentrations were assessed using Spearman rank correlation. Comparison of the distributions obtained by the 'TBM Clone B' and cultivar-screening datasets was made by the Kolmogorov-Smirnov (KS) test, with ties resolved by a jitter function to enable estimation of p-values. Ultimately, comparison between the sample preparation techniques was made by an equal variance t-test. Figures were produced using R and Vega-Lite (version 6.4.3). Additional figures such as histograms to evaluate the distribution of observations were included in the supplemental data. Missing observations resulting from incomplete sampling were not imputed, but were excluded only from analyses requiring paired observations.

3. Results

3.1 Grid dissection

Mescaline concentrations were quantified in 6×6 grid dissections of transverse stem sections from two *T. bridgesii* cultivars ('Experiment' and 'TCF1') (Fig. 1). The dissections yielded 30 and 31 analysed grid cells for each cultivar, with unsampled grid positions corresponding to areas outside the stem cross-section.

In 'Experiment', mescaline concentrations ranged from 1.34 to 25.31 mg/g. The highest concentrations occurred around the outer margins of the section, whereas the lowest values occurred within the central region. Although concentrations generally declined towards the interior, neighbouring grid cells frequently differed by several-fold, indicating appreciable fine-scale spatial variability.

The 'TCF1' cultivar exhibited a similar overall distribution, with mescaline concentrations ranging from 0.35 to 19.74 mg/g. Peripheral grid cells again generally contained the highest concentrations, whilst the lowest concentrations occurred within the central portion of the section. Compared with the 'Experiment' cultivar, mescaline concentrations were more uniform around the peripheral margin, although localised heterogeneity remained evident throughout the tissue.

Together, the two grid dissections demonstrate spatially heterogeneous mescaline distribution within the two *T. bridgesii* stems examined. Both specimens exhibited a broad radial pattern of higher concentrations towards the stem periphery and lower concentrations within the central tissue, alongside substantial variation among adjacent grid cells.

3.2 Vertical profiling

Mescaline concentrations varied substantially along mature *T. bridgesii* stems and between adjacent stems within the same planting (Fig. 2A; 2B). Chlorenchyma and parenchyma generally exhibited similar spatial patterns within individual stems, although localised differences between tissues were also observed.

In the fully profiled stems (Fig. 2A), the highest mescaline concentrations occurred at the basal sampling position for both cultivars. In 'Experiment', mescaline concentrations were relatively low throughout most of the sampled stem, with chlorenchyma ranging from 0.71 to 7.97 mg/g (median 1.71 mg/g) and parenchyma from 0.71 to 6.93 (median 2.13) mg/g. Three-point centred moving average (boxcar, CAR = 3) smoothing demonstrated a trend of increasing mescaline towards the basal end (Figure S1). Similarly, 'Nimbin' vertical chlorenchyma concentrations ranged from 0.04 to 7.26 (median 0.53) mg/g, whilst the parenchyma ranged from 0.08 to 9.88 (median 0.32) mg/g. Three-point centred moving average smoothing revealed a less consistent trend than in the 'Experiment' cultivar, although the most basal three-point window still gave the highest average.

Considering matched apical-basal measurements of chlorenchyma mescaline concentration (Fig. 2B), the three cultivars did not demonstrate uniform trends. 'Experiment' gave high basal concentrations in two of three matched samples (three of four, if including the stem with ten-points sampled). Basal samples of 'Nimbin' were lost, but the seven apical samples (incl. stem with ten samples) were highly variable with a range of 0.23 to 43.03 (median 17.00) mg/g; with the fully sampled stem yielding a higher basal concentration (one of one). However, 'TBM Clone B' gave higher basal concentration in only 1 of 3 stems. Together, of the eight stems that had paired apical-basal chlorenchyma measurements, five had higher basal concentrations. Evidently, neither the apical nor basal concentrations were consistently the highest ($p = 0.7266$, binomial sign two-tailed). Notwithstanding, some individual stems may have up to a 32-fold difference between the apical and basal concentration in extreme cases.

Adjacent stems taken from the same plant were noted to differ substantially in chlorenchyma mescaline concentration at comparable sampling positions. 'Experiment' basal positions differed 4.9-fold, whilst apical positions differed 2.8-fold (4 stems). 'Nimbin' apical positions differed 180-fold (5 stems). Accordingly, substantial heterogeneity was observed both vertically within stems and laterally between neighbouring stems.

3.3 Circumferential dissection

Mescaline concentrations were quantified in paired chlorenchyma and parenchyma samples from ten sequential rib panels around the circumference of two *T. bridgesii* cultivars ('Kai' and 'Nimbin') (Fig. 3). In both cultivars, chlorenchyma consistently contained higher mescaline concentrations than the corresponding parenchyma.

'Kai' exhibited relatively uniform chlorenchyma concentrations around the stem circumference, ranging from 18.88 to 27.65 mg/g, whilst parenchyma concentrations ranged from 7.77 to 11.64 mg/g. Likewise, 'Nimbin' showed only modest circumferential variation, with chlorenchyma concentrations ranging from 15.18 to 23.48 mg/g (one panel not analysed) and parenchyma concentrations ranging from 4.50 to 6.66 mg/g.

Unlike the pronounced radial gradients observed in the 6×6 grid dissections and the substantial vertical heterogeneity observed along stems, no obvious directional pattern associated with cardinal orientation was evident in either cultivar. Instead, mescaline concentrations remained comparatively consistent around the circumference of each stem. Within the two specimens examined, circumferential variation was smaller than the radial and vertical variation observed in the corresponding exploratory experiments; however, the limited biological replication precludes determining whether this pattern is generalisable across *T. bridgesii*.

3.4 Variation within 'TBM Clone B'

To assess the extent of intra-cultivar variation in mescaline concentration, *T. bridgesii* 'TBM Clone B' specimens from multiple gardens were analysed. Mescaline was detected in both chlorenchyma and parenchyma of all 'TBM Clone B' samples analysed (Fig. 4). The dataset comprised 25 paired tissue

measurements obtained from 22 plants representing multiple cultivated forms and seven Australian gardens, including repeated sampling of upper and lower stem segments from three individuals.

Mescaline concentrations in chlorenchyma ranged from 5.19 to 45.16 mg/g (median 29.04 mg/g; mean 27.32 ± 10.07 mg/g), whereas parenchyma concentrations ranged from 1.33 to 34.56 mg/g (median 18.16 mg/g; mean 17.58 ± 9.37 mg/g). Histograms for mescaline concentrations in the chlorenchyma (Figure S4) and parenchyma (Figure S5) appeared approximately symmetric on visual inspection, but analysts were not prepared to assume normality for subsequent tests on account of the small sample size. Chlorenchyma contained higher mescaline concentrations than the corresponding parenchyma in 22 of 25 paired samples (88%). This observation was significant, as chlorenchyma mescaline concentrations were higher than paired parenchyma concentrations (Wilcoxon signed-rank test, $v = 305, p = 0.000133$), with a median paired difference of 11.65 mg/g (IQR 3.00 to 14.78 mg/g).

3.5 Variation across cultivar screening panel

Mescaline concentrations were quantified in paired chlorenchyma and parenchyma tissues from 64 cultivated *T. bridgesii* specimens maintained under different horticultural cultivar names to examine tissue-specific distribution and the range of chemical variation across the cultivar screening panel (Fig. 5; Table 1). As most cultivars were represented by a single plant, individual measurements should not be interpreted as estimates of cultivar-level mean concentrations. Across the cultivar screening panel, mescaline was generally more concentrated in chlorenchyma than parenchyma, with mean concentrations 1.88-fold higher in chlorenchyma. Chlorenchyma concentrations ranged from 2.30 to 64.62 mg/g, whereas parenchyma concentrations ranged from 1.28 to 29.95 mg/g, representing an approximately 28-fold range in chlorenchyma concentrations among the specimens examined.

Table 1
Descriptive statistics of mescaline concentrations in chlorenchyma and parenchyma tissues across 64 cultivar-labelled *T. bridgesii* specimens.

Mean $\pm$ SD (mg/g)	Chlorenchyma	Parenchyma
	18.16 $\pm$ 13.51	9.66 $\pm$ 6.24
Median (mg/g)	14.60	8.35
Range (mg/g)	2.30–64.62	1.28–29.95

Across the cultivar screening panel, histograms for mescaline concentrations in the chlorenchyma (Figure S6) and parenchyma (Figure S7) both appeared positively skewed on visual inspection. Chlorenchyma concentrations tended to exceed parenchyma concentrations, as observed in 50 of 64 cultivar-labelled specimens. This tendency was significant, as mescaline was higher in chlorenchyma than paired parenchyma (Wilcoxon signed-rank test, $V = 1856, p < 0.001$). Chlorenchyma and parenchyma concentrations were moderately positively correlated across the cultivar screening panel (Spearman's ρ

= 0.56, $p < 0.001$), indicating that specimens with higher chlorenchyma concentrations generally also contained higher parenchyma concentrations.

To assess whether the variation observed across the cultivar screening panel exceeded the biological variation observed within a single cultivar, the distributions of mescaline concentrations in the cultivar-screening and 'TBM Clone B' datasets were compared. Adjacent violin plots of chlorenchyma mescaline concentrations in the cultivar-screening and 'TBM Clone B' datasets (Fig. 6) demonstrated that the cultivar-screening dataset had a lower central tendency than the 'TBM Clone B' dataset. Despite this, there was a notable upper tail of the cultivar-screening distribution where some specimens exceeded the highest value reported for the biological replicates of the single 'TBM Clone B' cultivar.

Whilst differences in the distribution were evident by inspection, a formal statistical evaluation was made. A null hypothesis was proposed that both datasets were drawn from the same distribution—that the variation observed across the cultivar-screening set did not exceed the biological variation observed among plantings of 'TBM Clone B'. A Kolmogorov-Smirnov (KS) test was applied to compare the distributions. Chlorenchyma concentrations of the cultivar-screening set were drawn from a different distribution from the 'TBM Clone B' dataset ($D = 0.51875$, $p = 0.000058$; no ties) resolved by jittering 'TBM Clone B' data, likewise the parenchyma of the respective sets were also drawn from different distributions ($D = 0.53063$, $p = 0.000033$; no ties). Inspection of empirical cumulative distribution functions (Figure S2 and S3) was consistent with the KS test results. However, this comparison should not be interpreted as demonstrating cultivar-level differences, because most cultivars in the screening panel were represented by a single plant and environmental, developmental and other sources of biological variation were not controlled. The analysis therefore demonstrates that the range and distribution of observations across the broader cultivar panel differed from those observed among the sampled 'TBM Clone B' plants, but cannot determine the extent to which cultivar identity contributed to that difference.

To aid cultivar comparisons, power calculations were performed assuming that the variation observed in 'TBM Clone B' was representative of within-cultivar variation for any other cultivars. For two-sample *t*-tests of equal sample size (two-tailed, $\alpha = 0.05$, 80% power), differences of 20, 10, or 5 mg/g (approximate effect sizes of 2.0, 1.0 and 0.5, respectively) would require sample sizes of 6, 17 or 65 per group, respectively). These estimates should be interpreted cautiously because within-cultivar variance may differ among cultivars and cultivation conditions, but they provide an initial guide for the design of future cultivar-comparison studies. Accordingly, the present screening data should be regarded as hypothesis-generating rather than as evidence ranking cultivar potency. Replicated sampling of individual cultivars across plants, gardens and cultivation conditions will be required to establish whether apparent differences are reproducible cultivar-level characteristics.

3.6 Recovery of mescaline following biopsy sampling

Mescaline concentrations for the chlorenchyma of six ribs were determined by the previously validated homogenisation sample preparation against the unvalidated needle biopsy with overnight soaking (Table 2).

Table 2
Descriptive statistics of mescaline concentrations in the ribs (n = 6) of a *T. pachanoi* cultivar, determined by the previously validated homogenisation sample preparation against the unvalidated needle biopsy with overnight soaking method; LCMS method is common.
*Significant decrease compared to validated sample preparation (P < 0.05, one-tailed, equal-variance t-test).

Mean ± SD (mg/g)	Validated (homogenised)	Unvalidated (soak)
	9.83 ± 1.55	7.74 ± 1.49*
Median (mg/g)	9.77	7.71
Range (mg/g)	8.06–11.80	5.89–11.80

Although the samples were matched by their nature in that each pair of data was from the same rib, circumferential variance was considered low. It was assumed that matched statistics would fail to control sufficient variance to justify the decrease in degrees of freedom compared to the unmatched test. This was evaluated by inspection of the scatter plot (Figure S8) and calculation of a Pearson R of -0.370, which demonstrated no meaningful positive correlation between the soaked biopsy plug and corresponding crushed specimen.

The extraction of mescaline by soaking the biopsied chlorenchyma in methanol only recovered 78.7% of the true amount determined by the validated sample preparation. This decrease of 2.1 mg/g (21.3%) was statistically significant ($t = 2.182, p = 0.027$, one-tailed). Whilst a decrease existed, it was small compared with the range of concentrations observed within ‘TBM Clone B’ and across the cultivar screening panel. Accordingly, indicative values measured by the minimally destructive biopsy may be suitable for some sample comparisons despite the negative bias, although the sampling method should be clearly identified when such values are reported.

4. Discussion

The present study substantially expands current understanding of mescaline distribution in *T. bridgesii*. Previous quantitative studies generally examined limited numbers of plants or cultivars and typically focused on outer cortical tissue [1, 18]. More recently, Berman et al. [17] compared mescaline concentrations among anatomically distinct radial tissue compartments—skin (epidermis and chlorenchyma), middle (parenchyma), and centre (pith)—in *T. pachanoi* with biological replication unspecified, whereas Lin et al. [16] examined longitudinal (apical-to-basal) spatial distribution within a single *T. pachanoi* stem using matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI). However, these studies have done little to address *T. bridgesii*. The present study integrates both radial tissue and longitudinal sampling strategies across a broad collection of cultivar-

labelled *T. bridgesii* specimens, providing a more comprehensive assessment of mescaline distribution within and among plants. By integrating radial, vertical, circumferential, replicated 'TBM Clone B' and cultivar-screening sampling, the present study demonstrates that mescaline distribution in *T. bridgesii* is heterogeneous across multiple biological scales. The exploratory spatial datasets indicated pronounced radial and vertical heterogeneity, whereas circumferential variation was comparatively limited in the two specimens examined.

Across multiple sampling strategies, chlorenchyma generally contained higher mescaline concentrations than adjacent parenchyma, consistent with an overall enrichment of mescaline in peripheral photosynthetic tissues. However, exceptions occurred in both the cultivar screening panel and individual plants, indicating that tissue identity alone does not fully determine mescaline localisation. Particularly striking was the degree of variation observed between neighbouring stems arising from the same planting, which in one cultivar differed by almost two orders of magnitude at comparable sampling positions. Substantial quantitative variation in specialised metabolites among individual plants is widely recognised and may arise from interacting genetic, developmental and environmental influences [19]. Such differences indicate that sampling a single stem may not adequately characterise the chemistry of an entire clone or planting.

Spatial position also contributed substantially to variation within individual plants. Although individual stems sometimes exhibited substantial differences between apical and basal tissues, a consistent directional gradient was not observed across the stems examined. This extends the observations of Lin et al. [16], who demonstrated longitudinal heterogeneity within a single *T. pachanoi* stem using MALDI-MSI, by showing that the direction and magnitude of longitudinal variation are not consistent across the *T. bridgesii* stems examined. Some stems contained considerably higher basal concentrations whereas others exhibited the opposite pattern. Consequently, sampling position along the stem can strongly influence measured mescaline concentration, but no universal apical-to-basal distribution was identified. In contrast, circumferential profiling showed comparatively little variation in the two plants examined. Together, these observations provide preliminary evidence that radial and vertical position may be important contributors to within-stem variation, whereas circumferential variation was comparatively limited in the specimens examined, although greater biological replication is required to establish the generality of this pattern. They also indicate that measurements from a single sampling position may not reliably represent an entire stem or plant.

These findings have important implications for phytochemical sampling and comparisons among analytical studies. Variation in sampling position and tissue selection may contribute substantially to differences in reported mescaline concentrations. We recommend that future studies explicitly report sampling position and tissue type, standardise sampling protocols where possible, and incorporate multiple sampling locations or biological replicates when estimating cultivar-level mescaline concentrations. More broadly, careful sample collection and preparation are recognised as critical determinants of metabolite composition and analytical reproducibility in phytochemical research [20]. The present findings suggest that standardisation should extend beyond tissue selection to include stem

developmental stage, longitudinal sampling position and the use of biological replicates. Circumferential orientation appeared comparatively unimportant in the specimens examined, whereas radial tissue composition and longitudinal position may substantially influence measured concentrations. Based on the present findings, a practical sampling strategy could involve collecting mature stems between the mid-point and near the apex (at least 150 mm from the apex), either as a whole transverse section or as a single rib or biopsy obtained from an arbitrary circumferential position. The comparison of biopsy sampling with the validated homogenisation method indicates that minimally destructive biopsy may be suitable for comparative studies, despite introducing a modest negative bias in measured concentration. Given that this bias was substantially smaller than the biological variation observed within and among plants, biopsy sampling may represent an acceptable compromise where preservation of valuable living specimens is a priority.

Considerable variation was also observed across the 64-specimen cultivar screening panel, with chlorenchyma concentrations differing by almost 30-fold. Chemically distinct phenotypes (chemotypes) are widely recognised in natural products research, illustrating that morphology alone may not reliably predict specialised metabolite composition and highlighting the importance of direct chemical characterisation [21]. 'TBM Clone B' has previously been reported to contain 0.48% mescaline by dry weight in outer cortical tissue [1], yet our screening demonstrated substantial variation among plants maintained under the same horticultural identifier and, in some cases, between upper and lower stem segments of the same plant. Cultivar identity alone is therefore insufficient to explain the observed variation. Comparison of the 'TBM Clone B' and cultivar-screening datasets indicated that the distribution of observations across the broader screening panel differed from that observed within the single replicated cultivar. However, cultivation conditions were not experimentally controlled, and environmental, developmental and other plant-specific factors may have contributed to the observed variation. Consequently, the present data cannot determine the extent to which differences among cultivar-labelled specimens reflect cultivar identity rather than these potentially confounding influences. The observed magnitude of intra-cultivar variation further illustrates why caution is required when interpreting single measurements. Based on the variance observed within 'TBM Clone B', relatively large concentration differences would be required before isolated observations could reasonably be interpreted as evidence of cultivar-level differences. Consequently, biological replication should be prioritised over increasing the number of cultivar-labelled specimens sampled. Developmental, architectural, environmental and genetic factors may contribute, although these were not investigated directly in the present study.

The observed anatomical and spatial variation also has implications for clinical and pharmacological research involving *Trichocereus* preparations. Where botanical materials are used in experimental or clinical settings, preparation methods that include different tissues or stem sections may produce preparations with differing mescaline concentrations, even when derived from the same plant. Consequently, differences between preparations may reflect both preparation methodology and underlying biological heterogeneity. These findings highlight the importance of documenting tissue composition, developmental stage and sampling strategy when interpreting or comparing studies

involving *Trichocereus*-derived materials and underscore the need for careful standardisation of botanical materials in future pharmacological and clinical investigations.

The findings also have implications for horticultural selection, natural product development and harm reduction. The substantial variation observed among plants maintained as 'TBM Clone B', together with spatial variation within individual plants, indicates that cultivar name or a single analytical measurement should not be treated as a reliable predictor of mescaline concentration. Where identification of particular chemotypes is desirable, candidate cultivars should therefore be evaluated using replicated measurements from standardised sampling positions and, ideally, across cultivation environments. Such selection could include low-mescaline chemotypes for applications where reduced mescaline content is preferred, including to minimise the potential for diversion to psychoactive use, or high-mescaline chemotypes for natural product development where consistent mescaline-rich botanical material is required. More broadly, reliable characterisation of mescaline content may support harm-reduction approaches by reducing uncertainty regarding the potency of botanical materials.

Several limitations should be acknowledged. Only cultivated Australian-grown material was examined, rather than wild populations, and environmental variables were not experimentally controlled. The study evaluates plants maintained in Australian horticultural collections as *T. bridgesii*, with cultivar identities based on horticultural provenance rather than authentication via genetic analyses or comparison with herbarium voucher specimens. Whilst the provenance and labelling of the study material provide a reasonable level of confidence in these horticultural identities, occasional misidentification or label drift cannot be excluded. Future studies incorporating genetically authenticated material would enable more robust verification of cultivar identity and enable direct assessment of the relationship between genotype and mescaline chemotype. Biological replication was also limited for several components of the study. Most cultivars in the screening panel were represented by a single plant, the grid and circumferential investigations examined only two specimens each, and vertical profiling was undertaken in a limited number of stems representing three cultivar labels. Consequently, the cultivar screening characterises variation among the specimens examined rather than providing estimates of cultivar-level mean concentrations, while the spatial investigations should be regarded as exploratory evidence of patterns requiring confirmation in larger numbers of plants. Most sampling also represented single time points, preventing assessment of seasonal or temporal variation. Future research should investigate the physiological mechanisms underlying mescaline localisation and the contribution of seasonal, developmental, environmental and genetic factors. Similar multiscale sampling of other mescaline-containing cacti would clarify whether the spatial patterns observed in *T. bridgesii* are conserved across the San Pedro complex.

5. Conclusion

This study provides a multiscale assessment of mescaline distribution in cultivated *T. bridgesii*, identifying substantial variation among sampled plants, between tissue types and within individual stems. Mescaline was generally more concentrated in peripheral chlorenchyma than in parenchyma, but

this relationship was not universal. Exploratory spatial sampling identified pronounced radial and vertical heterogeneity, whereas circumferential variation was comparatively limited in the two plants examined. The cultivar screening panel additionally identified a wide range of mescaline concentrations among cultivar-labelled specimens, but limited biological replication and uncontrolled cultivation conditions preclude attributing these differences conclusively to cultivar identity.

These findings indicate that mescaline concentration measured at a single sampling position may not reliably represent an entire stem or plant. Tissue selection, sampling location and within-cultivar variation should be considered when investigating mescaline concentrations in *T. bridgesii*. More detailed reporting of tissue type and sampling position, together with greater standardisation of plant sampling, would improve comparability among future studies. Future studies should investigate the genetic, developmental, environmental and seasonal factors underlying spatial and within-cultivar variation in mescaline concentration.

Declarations

Declaration of competing interest

Liam Engel is founder of The Mescaline Garden educational nursery. The other authors declare no competing interests.

Declarations

Competing Interests

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Data availability

The datasets generated and analysed during the current study are not publicly available because some data are associated with plant material and provenance information provided by third-party contributors. De-identified data supporting the findings of this study are available from the corresponding author upon reasonable request, subject where applicable to contributor permissions.

Author Contribution

L.E.: Conceptualisation, Methodology, Validation, Investigation, Formal analysis, Resources, Writing – original draft, Visualisation. D.R.H.: Methodology, Validation, Formal analysis, Investigation, Writing – review and editing. P.G.: Methodology. D.S.: Investigation. C.G.L.: Writing – review and editing, Supervision. M.L.: Conceptualisation, Methodology, Writing – review and editing, Supervision, Project administration.

Acknowledgement

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Figures

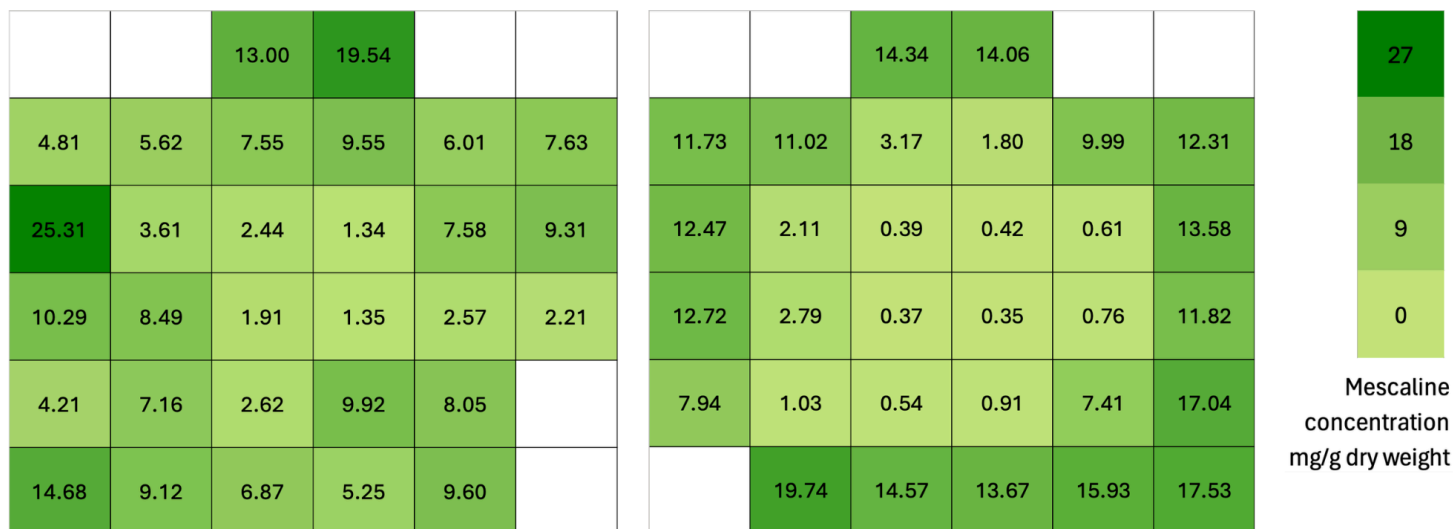


Figure 1

Figure 1. Spatial distribution of mescaline concentrations (mg/g) across 6 × 6 grid dissections of transverse stem sections from two *T. bridgesii* cultivars ('Experiment' left, 'TCF1' right). Each square represents an individual grid sample. White squares indicate positions outside the stem where no tissue was collected. Both heat maps use the same colour scale (far left). Blank cells indicate grid positions that were not sampled.

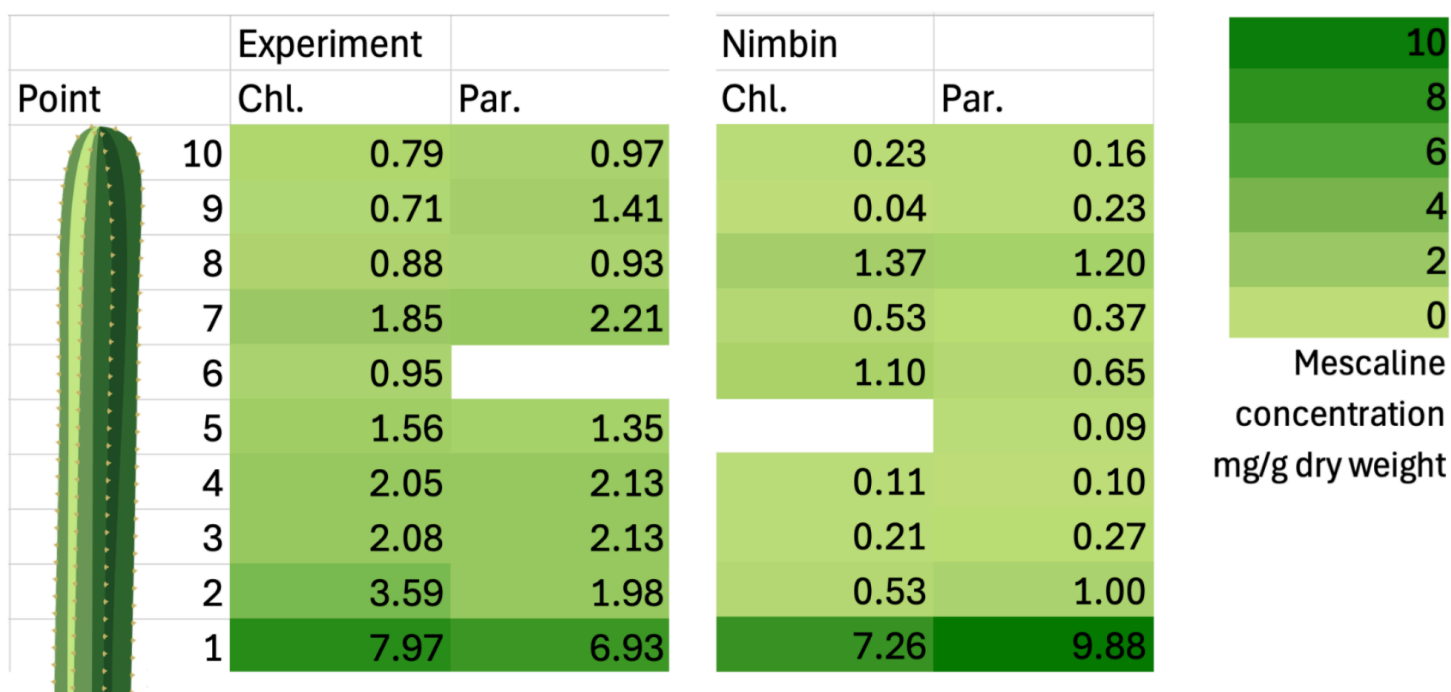


Figure 2

Figure 2A. Mescaline concentrations (mg/g) in chlorenchyma and parenchyma tissues sampled vertically from mature *T. bridgesii* stems. Point 10 represents the apical sampling position approximately 20 cm below the apex, with points progressing towards the stem base (Point 1). Experiment’ (left); ‘Nimbin’ (right). Colour scale (far left). Blank cells indicate positions that were not sampled.

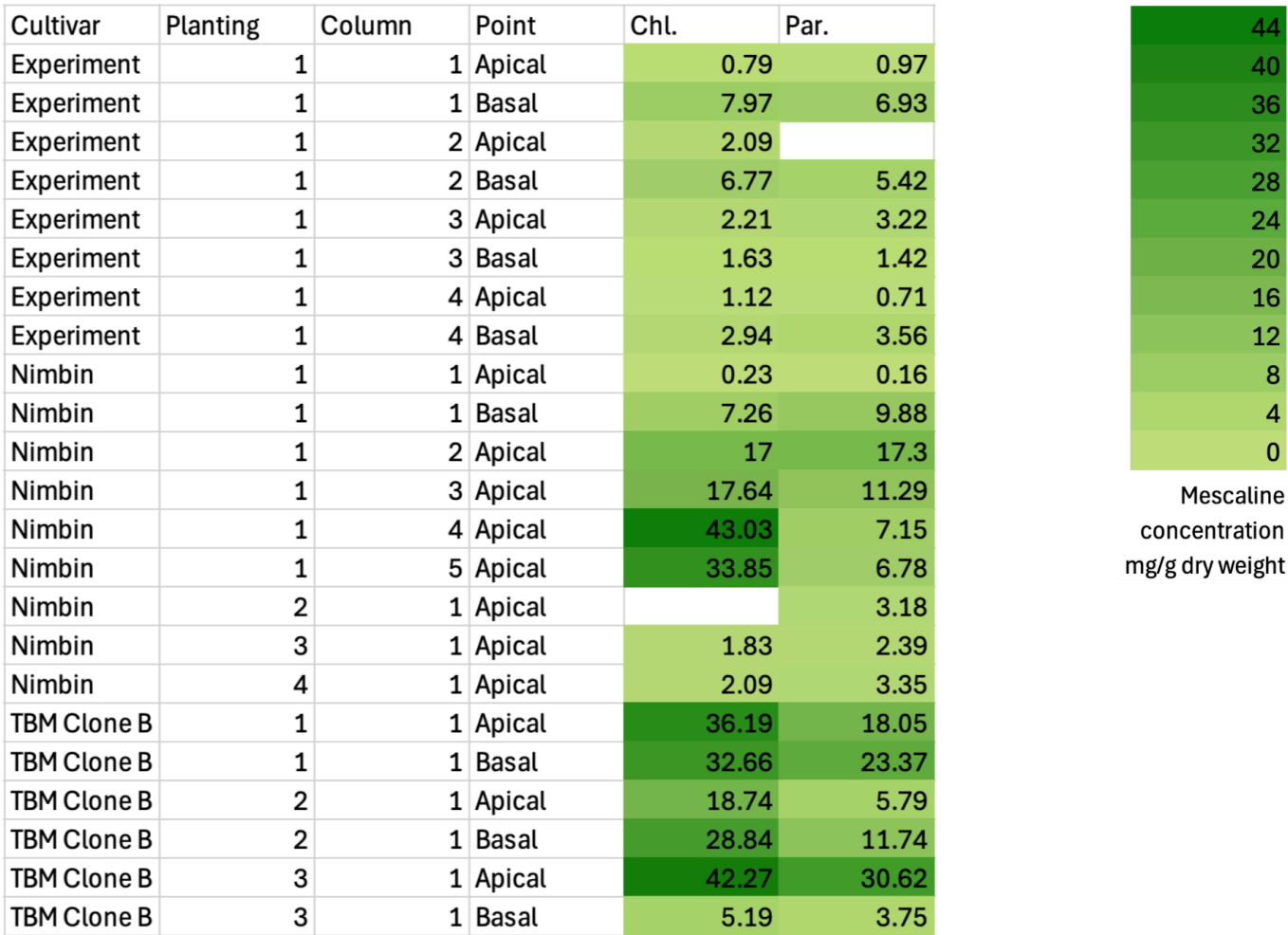


Figure 3

Figure 2B. Mescaline concentrations (mg/g) in additional measurements obtained from offsets and mature stems in other ‘Nimbin’ and ‘Experiment’ plantings using apical (Point 10) and basal (Point 1) sampling positions, and ‘TBM Clone B’ upper and lower sampling positions. ‘Nimbin’ and ‘Experiment’ determinations by minimally destructive needle biopsy, ‘TBM Clone B’ determinations by homogenisation and subsampling. Colour scale (far left). Blank cells indicate positions that were not sampled.

		Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Panel 7	Panel 8	Panel 9	Panel 10
Kai	Chlorenchyma	23.29	22.26	25.16	23.92	20.68	18.88	22.45	21.45	25.73	27.65
	Parenchyma	8.97	11.04	11.17	11.64	11.59	9.05	9.45	7.85	7.77	9.91
Nimbin	Chlorenchyma	15.18	16.96	15.24	16.29	19.64	N/A	23.48	17.22	16.41	16.15
	Parenchyma	4.76	5.85	6.66	6.16	5.99	5.13	4.5	5.37	6.19	6.37

Figure 4

Figure 3. Mescaline concentrations (mg/g) in paired chlorenchyma and parenchyma tissues from ten sequential rib panels around the circumference of two *T. bridgesii* cultivars ('Kai' and 'Nimbin'). Panels numbered 1–6 in an anticlockwise direction, with the junction between 1 and 2 oriented north.

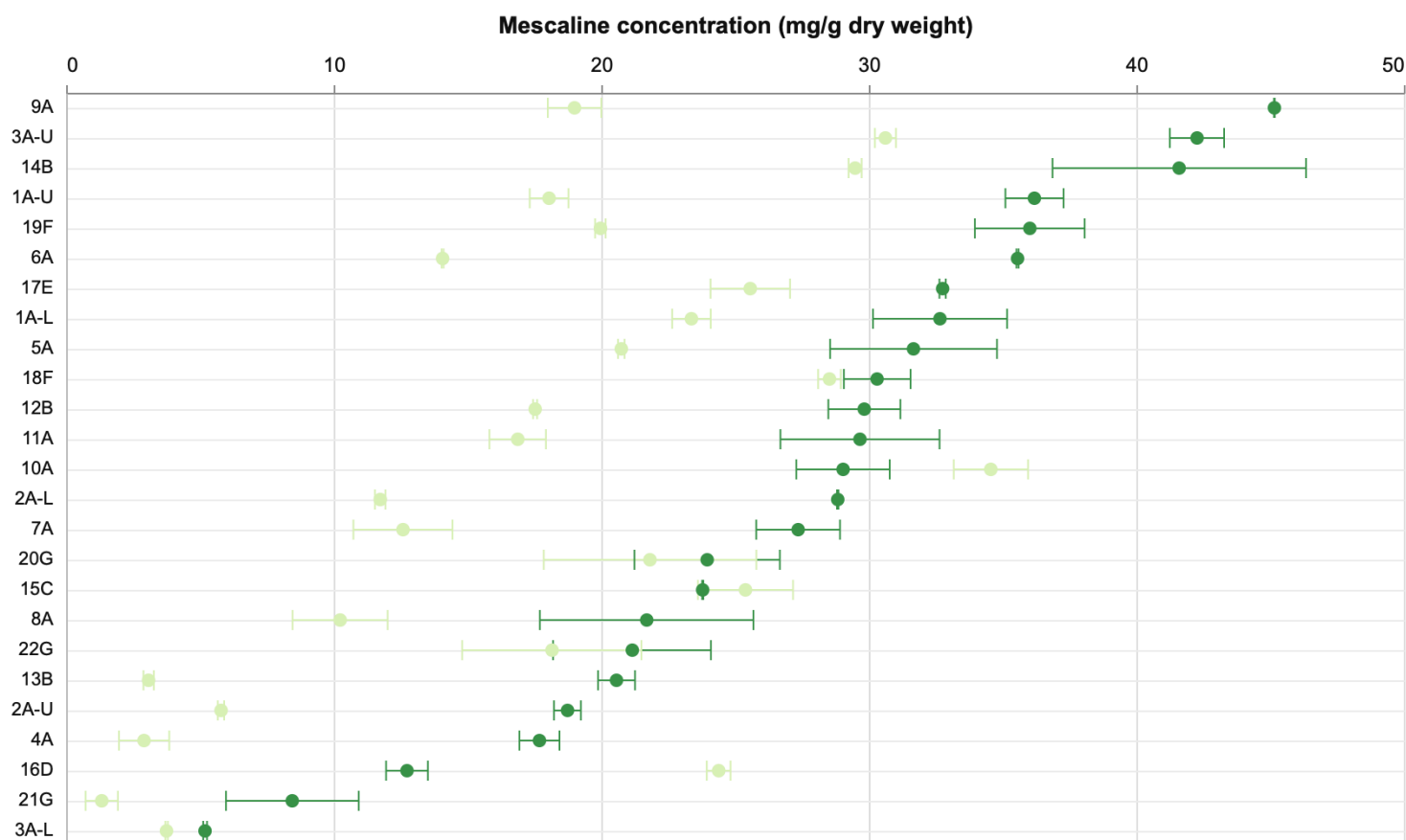


Figure 5

Figure 4. Mescaline concentrations (mg/g) in paired chlorenchyma and parenchyma tissues of 25 *T. bridgesii* 'TBM Clone B' samples. Points represent the mean mescaline concentration from duplicate independent sample preparations (two separately extracted aliquots prepared from the same homogenised biological sample), and error bars indicate the minimum and maximum values of the two preparations. Samples are ordered by descending chlorenchyma concentration. Sample labels comprise the plant number followed by the garden identifier. Where two stem segments were analysed from the same plant, suffixes -A and -B denote the apical and basal stem segments, respectively.

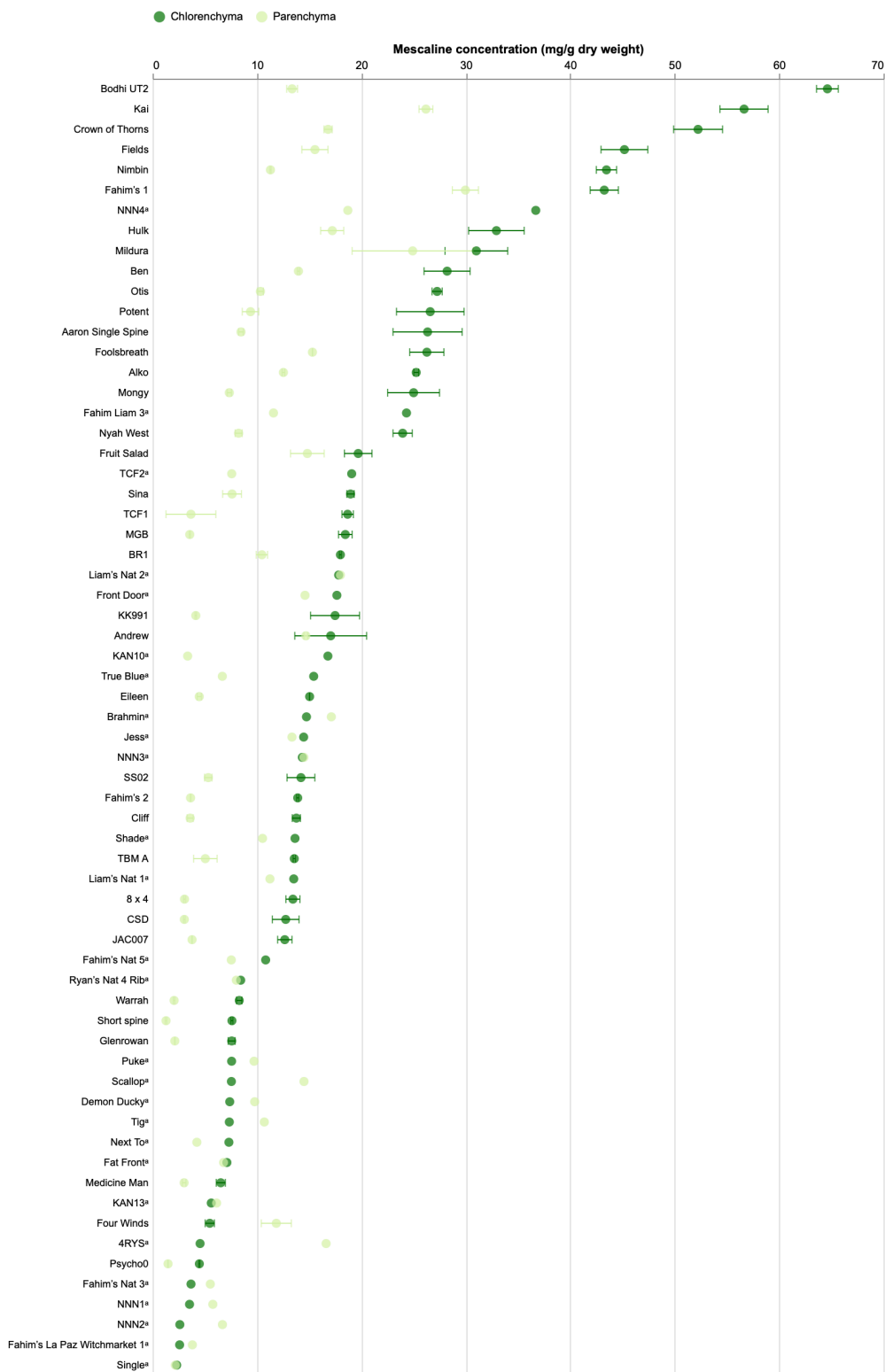


Figure 6

Figure 5. Mescaline concentrations (mg/g) in paired chlorenchyma and parenchyma tissues of 64 cultivar-labelled *T. bridgesii* specimens. Points represent the mean mescaline concentration from duplicate independent sample preparations (two separately extracted aliquots prepared from the same homogenised biological sample), and error bars indicate the minimum and maximum values of the two preparations. Cultivars marked with superscript a were analysed from a single soaked needle biopsy

preparation owing to limited sample availability; duplicate independent sample preparations were therefore not possible.

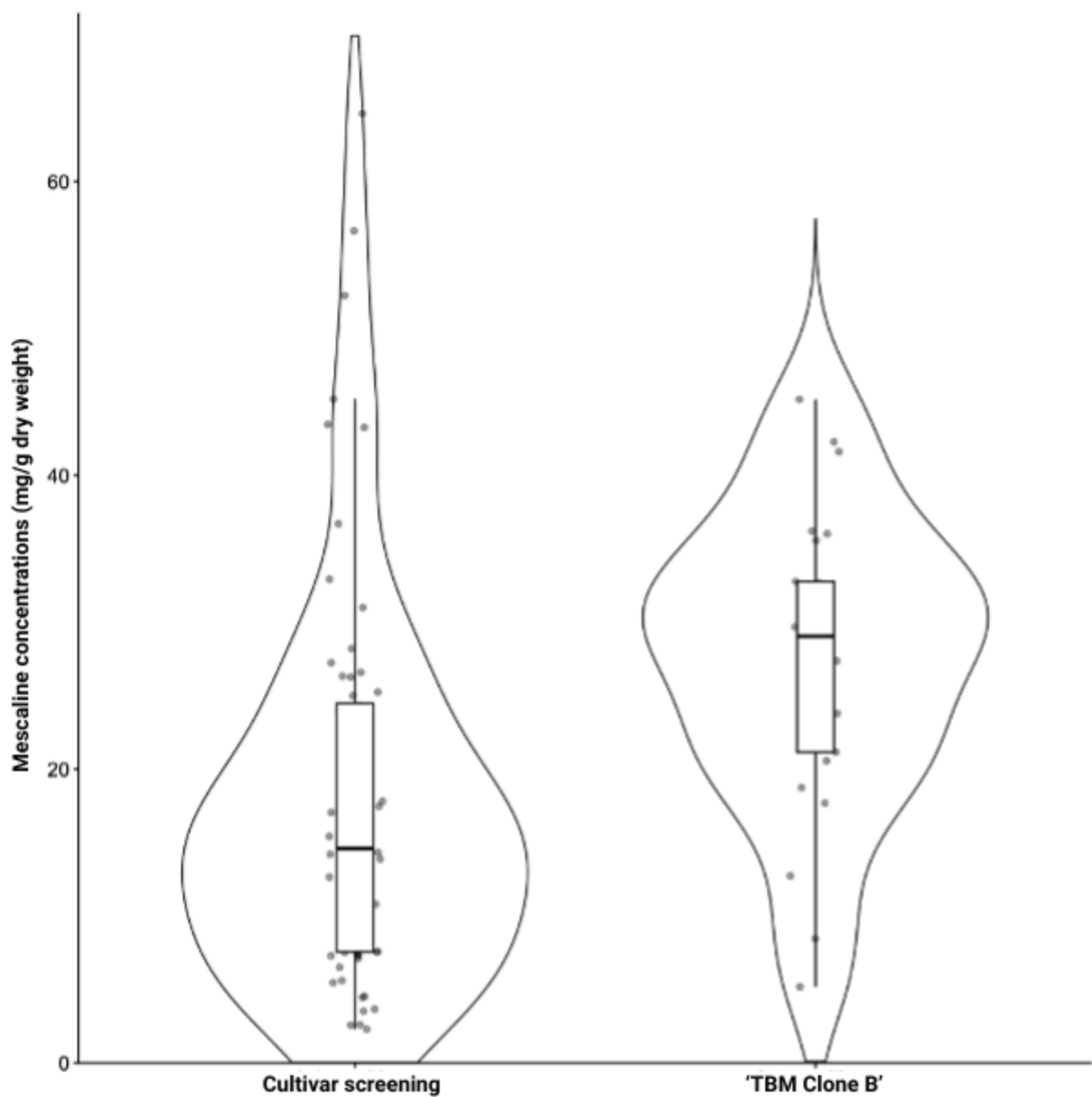


Figure 7

Figure 6. Violin plots to summarise the distributions of chlorenchyma mescaline concentrations in the cultivar-screening (n = 64) and 'TBM Clone B' (n = 25) datasets.

Supplementary Files

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