1 ***De-novo* draft genome sequence of *Crocus Sativus* L, Saffron, a golden condiment.**

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8 9 **Abstract:**

10 *Crocus sativus* L, saffron is the highest priced but low yielding plant of medicinal and culinary im-
11 portance. Despite its economic status, the omic information on this plant is very scarce, with only a
12 couple of transcriptomics and epigenetic studies. In the present study, the draft genome sequence of
13 *C. sativus* has been assembled using Illumina sequencing. In fact, this is the first genome sequence
14 from any member of family *Iridaceae*. Genome size of *C. sativus* was estimated to be 3.5 Gb and
15 the draft genome is 3.01 Gb long with 84.24% genome coverage. In total, 8,62,275 repeats and
16 9,64,231 SSR markers have been identified. A total of 53,546 functional genes were annotated, out
17 of which, 43,649 proteins were associated with GO annotation. 5726 proteins were identified as
18 transcription factors, with MYB & MYB related family proteins being more abundant. Orthology
19 analysis of *C. sativus* with 3 different monocot species of the same plant order and rice (model
20 monocot plant) revealed 7328 proteins clusters to be conserved in all the five plant species, whereas
21 2510 proteins cluster were unique to *C. sativus* only. 10,912 unigenes of *C. sativus* were mapped to
22 387 KEGG pathways of monocot. The genes involved in the pathway of apocarotenoids biosynthe-
23 sis (crocin, crocetin, picrocrocin, and safranal) were present in the draft genome.

24 **Keywords:** *Crocus sativus*, *denovo* genome assembly, apocarotene biosynthesis pathway, MYB
25 Tfs, SSR markers, Orthology analysis.

26 27 **Introduction:**

28 Plant genomics, with the increasing number of whole genome sequences available, has unlocked the
29 genetic treasures, that would be impossible in absense of the genome sequence. Though second and
30 third generation sequencing technologies, coupled with ever advancing bioinformatic tools/pipe-
31 lines, have made the sequencing of complex and huge genomes economical and easy, but still till
32 date there are only approximately 1565 plant genome sequenes available in databanks
33 (NCBI:<https://www.ncbi.nlm.nih.gov/assembly>) . Some of the recently sequenced and assembled

34 plant genome are rice (Choi et al., 2020), Maize (Liu et al., 2020), Asparagus (Harkess et al.,
35 2017), Wheat (Alonge et al., 2020) and tea (Xia et al., 2020) etc., however the genome of the plants
36 belonging to *Crocus* genera or *Iridaceae* family, have not reported so far.

37 Saffron (*C. sativus*) referred as ‘Golden Condiment’ is world’s most expensive spice costing about
38 70,000 INR/pound, with medicinal properties and cosmetic uses (Mzabri et al., 2019; Magotra et
39 al., 2021). More than 150 volatile and aroma-yielding compounds contribute to the flavor, color,
40 and aroma of the Saffron spice, wherein the main chemical constituents in the stigma of saffron are
41 crocin, crocetin, picrocrocin, and safranal (Samarghandian et al., 2014; Maggi et al., 2020). *C.*
42 *sativus* is an autumn-flowering perennial sterile triploid plant ($2n = 24$) with, ~3.5 Gb haploid
43 genome (Brandizzi and Caiola 1996, 1998). Being sterile, it fails to produce viable seeds and repro-
44 duces vegetatively by underground corms and is reported to lack genetic variation. Various molecu-
45 lar markers (RAPD, ISSR, AFLP, SSR microsatellites) and epigenetic approaches have suggested
46 the existence of limited genetic variability (Rubio-Moraga et al., 2009; Siracusa et al., 2013; Bus-
47 coni et al., 2018; Mir et al., 2021). To discover authentic genetic markers, mining genes for sec-
48 ondary metabolites and improvement of breeding, sequencing of its genome was the only alterna-
49 tive. In addition, it’s ancestry is also controversial (Alsayied et al., 2015; Nemati et al., 2019), that
50 could be also settled, if its complete genome sequence is available.

51 Hybrid sequencing approaches, comprising of second and third generation sequencing technologies,
52 have facilitated sequencing of complex genomes economically. Illumina sequencing technology is
53 preferred for first sequencing attempt, as it generates good sequencing data for better genome cov-
54 erage and has low error rate as compared to third generation sequencing technologies (Edwards and
55 Batley 2010). In the present study, *C. sativus de novo* draft genome sequence has been assembled
56 using short read Illumina sequencing technology. The draft genome sequence unravels the putative
57 markers, metabolic pathways, transcription factors and orthologous genes.

58 **Materials and Methods:**

59 **Sample collection, genome size estimation, whole genome library preparation and Sequencing:**

60 *C. sativus* corms were collected from Kishtwar, J&K (33.3116° N, 75.7662° E) in 2019. Corms
61 were grown in the pots for period of three months and leaves were harvested for genome size esti-
62 mation. Genome size of the plant was estimated by flow cytometric (Hare and Johnston 2011) and
63 k-mer based method using JellyFish (Marçais and Kingsford 2011). Genomic DNA was extracted
64 from corm tissue using CTAB method (Rogers and Bendich 1994) and quality and quantity was ac-
65 cessed using Qubit (Invitrogen) and agarose gel electrophoresis. 3 ug DNA was used to construct
66 WGS DNA libraries with 550bp and 800bp insert sizes using NEB next Ultra DNA Library Prepa-
67 ration Kit according to the Illumina's protocol. Quality of the libraries were evaluated using Tapes-

68 tation (Agilent 4200) and Qubit HS DNA Assay Kit (Invitrogen). Sequencing was done on the
69 HiSeqX platform (150-bp paired-end (PE) reads) to generate 321 Gb data (~92X coverage).

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71 **De-novo Genome Assembly and statistics:** Quality of raw reads was evaluated using FastQC tool
72 (Andrews S. 2010) and low quality bases ($<q30$) and sequencing adapters were removed using trim-
73 momatic software (Bolger et al., 2014). De-novo genome assembly was performed using two as-
74 semblers namely Soapdenovo2 (Luo et al., 2012) and MaSuRCA (Zimin et al., 2013). Soapdenovo2
75 assembly was executed using different kmers (73 kmer predicted by KmerGenei along with 69, 71
76 kmers) (Chikhi and Madvedev 2013). The statistics of soapdenovo2 assemblies were compared to
77 select the better assembly that was designated as Cs_Assembly_1. MaSuRCA assembly was done
78 using the raw reads and was designated as Cs_Assembly_2. The quality of assemblies was ac-
79 cessed using BUSCO against Viridiplantae lineage from OrthoDB database (Simao et al., 2015).
80 Subsequently, raw illumina reads were mapped back to Cs_Assembly_2 using Bowtie2 (Langmead
81 2010) and previously published transcriptome data (Baba et al., 2015; Jain et al., 2016) and mapped
82 to Cs_Assembly_2 using BWA (Li and Durbin 2010). Repetitive regions in Cs_Assembly_2 was
83 identified using Repeatmasker and GenomeScope v2 (Ranallo-Benavidez et al., 2020, Smit et al.,
84 2015) and SSR markers were identified using MISA (Beier et al., 2017).

85 **Genome prediction and annotations, orthology and metabolic pathway analysis:** Cs_Assem-
86 bly_2 was further analysed for gene prediction using the MAKER (Campbell et al., 2014) wherein
87 *C. sativus* transcriptome data was used as EST evidence (Jain and coworker 2016), Viridiplantae
88 database (UNIPROT) as protein evidence, maize as Augustus gene prediction model and *Oryza*
89 *sativa* as snap hmm. Predicted proteins were further annotated using BLASTp against NR (NCBI)
90 and viridiplanteae (UNIPROT) database with modified parameters (E-value- $1e^{-3}$, sequence identity
91 $>40\%$ and query coverage $>70\%$). Annotated proteins were analysed for GO annotations against bi-
92 ological processes, cellular component and metabolic processes using WEGO (Jia et al., 2018).
93 Transcription factors (Tfs) proteins were identified against PlantTFDB (Jinpu et al., 2017) using
94 BLASTp with the modified parameters (E-value- $1e^{-3}$, sequence identity $>30\%$, query coverage
95 $>70\%$). Orthologous genes were compared with *Asparagus officinalis*, *Dendrobium catenatum*,
96 *Phalaenopsis equestris*, *Apostatia shenzhenica* of the same plant order along with *Oryza sativus*
97 (Rice) using Orthovenn2 (Xu et al., 2019) The proteins sequences of all the plants were downloaded
98 from Phytozome database (David et al., 2012). Various metabolic pathways in *C. sativus* genome
99 were analysed using KAAS webserver (Kyoto Encyclopedia of Genes and Genomes Automatic An-
100 notation Server) (Moriya et al., 2007).

101 **Data availability:** Whole genome sequencing raw reads has been submitted to NCBI SRA under
102 bioproject PRJNA734464. The draft genome of *Crocus sativus* has been submitted in SRA under

103 bioproject PRJNA739096. All the processed data including draft genome, annotated proteins, SSR,
104 Transcription factors, and supplementary tables and figures can be accessed at [http://caps.ncb-](http://caps.ncb-s.res.in/download/csat)
105 [s.res.in/download/csat](http://caps.ncb-s.res.in/download/csat).

106 Results and Discussion

107 *Crocus sativus* genome, being reported here, is the first draft genome sequence of the plant belong-
108 ing to the *Iridaceae* family. Genome size of *C. sativus* was estimated to be 3.5 Gb (3,578,575,507
109 bases), using flow cytometry and kmer method. Genome size estimated was comparable to earlier
110 reports, wherein it was estimated to be 3.44 Gb using flow cytometry being grown in Italy, Spain
111 and Israel (Brandizzi and Cajola 1997; 1998). On the basis of size of the genome, 321 Gb WGS
112 data of *C. sativus* was generated, with an overall coverage of ~92X using Illumina sequencing (Ta-
113 ble 1). *De novo* genome assembly and annotation of *C. sativus* was performed using the bioinfor-
114 matics pipeline represented in Fig 1 for easy comprehension.

115 **De-novo genome assembly:** *C. sativus* genome was *de-novo* assembled using SOAPdenovo2 (Luo
116 et al., 2012) and MaSuRCA softwares (Zimin et al., 2013). Soapdenovo2 assembly with kmer 71
117 was comparatively better than other two kmers (69 and 73) and was designated as Cs_Assembly_1
118 with N50 value of 1596 and 77.9% genome coverage (Table 2). De-novo assembly with MaSuRCA
119 was designated as Cs_Assembly_2 with N50 value of 1860 and 84.24% genome coverage. Cs_As-
120 sembly_2 was found comparatively better than Cs_Assembly_1 as the assembly statistics, such as
121 N50, largest scaffold, genome coverage and BUSCO completeness were higher in Cs_Assembly_2
122 than Cs_Assembly_1. (Table 2). Further, ~87.28% of raw reads mapped back to Cs_Assembly_2,
123 thereby indicating that most of data has been utilized for genome assembly. In addition, two previ-
124 ously published transcriptome data sets (Jain et al., 2016; Baba et al., 2015) were mapped to the
125 Cs_Assembly_2 and mapping percentage of 99.92% and 92.02% were observed against Cs_Assem-
126 bly_2 (Table 3). High mapping percentage represented the presence of most of the reported exons/
127 CDS in the Cs_assembly_2 even though the genome assembly was fragmented with less N50 value.
128 *Polygonum cuspidatum* genome was de-novo assembled using Soapdenovo2 with Illumina reads
129 and generated an assembly of 2.56 Gb, with N50 value of 3215 and 98.5% genome coverage
130 (Zhang et al., 2019). Similarly, the genome of *Linum usitatissimum*, flax plant was *de novo* assem-
131 bled using Illumina reads having N50 scaffold of 694 Kb with 81% of genome coverage (Wang et
132 al., 2012). Genome coverage of *C. sativus* was comparatively more than flax genome but less than
133 *Polygonum cuspidatum* genome using same sequencing technologies.

134 **Repeats and SSR markers:** Total repeats length in *C. sativus* genome (Cs_assembly_2) was
135 1,460,908,750 bp (40.8%) as predicted by GenomeScope version 2. A total of 8,62,275 repeats
136 were identified in Cs_assembly_2 wherein simple repeat (48.41%) and LTR (30.34%) were the
137 most abundant in the genome. Specifically, Copia & Gypsy were the most abundant LTR repeats

(Table 4). A total of 9,64,231 SSR markers were identified in Cs_assembly_2 wherein monomeric SSR repeats (4,86,140- 50.4%) were more abundant as compared to dinucleotide (2,94,819 - 30.5%) and trinucleotide repeats (1,46,991- 15.2%) with “A”, “TA”, “TTG” most abundant SSRs in each groups. The abundance of Tetranucleotide (15,375-1.59%), pentanucleotide (8596-0.9%) and hexanucleotides (12,310-1.27%) repeats each was less than 2% of total SSRs with “AAAT”, “TATAT” and “TAACCC” most abundant in respective SSRs (Table 5). SSR markers are reported to be multi-allelic, relatively abundant, widely dispersed across the genome and have been used in genetic diversity analysis, parentage assessment, species identification and mapping genetic linkage (Feng et al., 2016). These markers can be further evaluated for their application in *C. sativus*. Earlier studies on *C. sativus* transcriptome has reported the presence of 16,721 SSRs (Jain et al., 2016) and 79,028 SSRs (Qian et al., 2019) using transcriptome analysis, but higher number of SSR (9,64,231) were discovered in the present study based on genome sequence.

Gene prediction and annotation: In total 2,54,038 proteins were predicted from Cs_assembly_2 using MAKER pipeline. A total of 52,435 and 52,545 proteins were annotated based on BLASTp against NR and viridiplanteae database respectively (Table 6). BUSCO analysis revealed the presence of 75.7% of the plant conserved genes/orthologues in the *C. Sativus* genome. Out of total proteins, 51% (26796) were annotated to 8 top-hit plant species (Fig 2). Maximum number of proteins were annotated against *Asparagus officinalis* (9213) indicating *C. sativus* to be phylogenetically closer to *Asparagus officinalis*, as both the plants belong to same plant order Asparagales (Fig 2). 85% of total proteins (43,649) were associated with gene ontology (GO) ids and classified into biological processes (BP: 22,092 proteins) abundant in cellular and metabolic processes, cellular components (CC: 24,399 proteins) mostly localised in cell and organelle parts and molecular functions (MF: 34,442 proteins) most abundant in catalytic and transporter activities (Table S1).

Transcription factors: Out of the total annotated proteins, 5726 unique *C. sativus* proteins were identified as transcription factors (TFs) belonging to 57 TFs families. MYB & MYB related family proteins (11.86%), being more abundant Tfs, followed by bHLH, C2H2, NAC, FAR1, C3H, ERF, bZIP, WRKY and B3 were the top 10 abundant transcription factors family proteins (Fig 3, Table S2). TFs like MYB & MYB related, bHLH, WRKY are reported to regulate secondary metabolite (apocarotenoid) biosynthesis in *C. sativus* (Jain et al., 2016). Earlier reports on *C. sativus* transcriptome has identified less number of TFs (3819, 2601), whereas the most abundant Tfs family remains same (Jain et al., 2016; Baba et al., 2015).

Comparative genomics: *C. sativus* annotated proteins (52,545) was compared with 3 monocots plants of same order, whose genome and annotations were available in Phytozome database (David et al., 2012), namely *Asparagus officinalis*, *Phalaenopsis equestris*, *Apostatia shenzhenica* along

172 with a model monocot plant *Oryza sativus* (Rice) using Orthovenn2. A total of 23,744 proteins cluster
173 ter were found in all the plants wherein 21,606 were orthologous clusters that were atleast common
174 in two species and 2138 were single copy gene clusters wherein each cluster have only one gene
175 from each plant species. Conservation of 7328 proteins clusters, comprising of 51,803 proteins,
176 was observed among the five species (*C. sativus*:10,001 proteins, *A. officinalis*:9552, *P. equestris*:9012,
177 *A. shenzhenica*: 8570 and *O. sativa*:14,668) (Fig 4a and 4b). The conserved proteins
178 clusters were found to be associated with biological processes (BP-23,010 proteins), cellular component
179 (CC-582 proteins) and molecular functions (MF-957 proteins) and were enriched in defence
180 response, RNA modification, DNA integration, regulation of transcription, rRNA processing and
181 protein phosphorylation (Supplementary table S3). However, 2510 protein clusters (7914 proteins)
182 were unique to *Crocus sativus* only, out of which 1636 clusters (4595 proteins) were associated with
183 slimmed GO terms (BP: 5201, CC: 63, MF:303 proteins) associated with nucleic acid binding,
184 transferase, hydrolase, oxidoreductase activity and protein and DNA binding activity (Supplementary
185 Table S3). As per orthology analysis also, *C. sativus* was found phylogenetically closer to *A. officinalis*
186 as more protein clusters were orthologous between *Crocus sativus* and *Asparagus officinalis*
187 than to other plants compared in the study (Fig 5).

188 **Metabolic pathway analysis:** A total of 10,912 *C. sativus* proteins were mapped to 395 KEGG
189 pathways of monocots. Various pathways like carbohydrate metabolism, energy metabolism, lipid
190 metabolism, nucleotide metabolism, amino acid metabolism, glycan metabolism, metabolism of co-
191 factors and vitamins along with biosynthesis of terpenoids, polyketides and other secondary metabolites
192 were found complete wherein all the genes involved in pathway were present in draft assembly
193 (Table S4). We, further, investigated the presence of genes involved in the synthesis of apocarotenoids
194 namely crocins, picrocrocins, and safranal that are produced in the stigma of *C. Sativus*.
195 These apocarotenoids impart red color, bitter taste, and pungent aroma to stigma of saffron and have
196 various medicinal properties (Maggi et al., 2020). The molecular basis of apocarotenoid biosynthesis
197 in *C. Sativus* has been well studied using transcriptomics studies (Baba et al., 2015; Jain et al.,
198 2016). In the present study, the genes encoding the enzymes involved in carotene biosynthesis pathway,
199 regulating the apocarotenoids synthesis, were present in the *C.sativus* genome (Fig 6). This is
200 the first de novo draft genome sequence of *Crocus sativus* that needs to be complemented with the
201 long read sequencing technology (PacBio) to fill in the gaps in the present genome to generate a
202 complete genome sequence. However this draft genome sequence, in addition to revealing previous
203 unknown genomic information on saffron, will also be used as a reference genome for future
204 genome sequencing attempts in saffron.

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210 References

- 211 1. Alonge M, Shumate A, Puiu D, Zimin AV, Salzberg SL. 2020. Chromosome-Scale Assem-
212 bly of the Bread Wheat Genome Reveals Thousands of Additional Gene Copies. *Genetics*.
213 Oct;216(2):599-608. doi: 10.1534/genetics.120.303501. Epub 2020 Aug 12. PMID:
214 32796007; PMCID: PMC7536849.
- 215 2. Alsayied NF, Fernández JA, Schwarzacher T, Heslop-Harrison JS. 2015. Diversity and rela-
216 tionships of *Crocus sativus* and its relatives analysed by inter-retroelement amplified poly-
217 morphism (IRAP). *Ann Bot*. 2015 Sep;116(3):359-68. doi: 10.1093/aob/mcv103. Epub 2015
218 Jul 1. PMID: 26138822; PMCID: PMC4549961.
- 219 3. Andrews S. 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data.
220 Available online: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>
- 221 4. Baba SA, et al. 2015. Comprehensive transcriptome analysis of *Crocus sativus* for discovery
222 and expression of genes involved in apocarotenoid biosynthesis. *BMC Genomics* 16, 698
223 (2015).
- 224 5. Beier S, Thiel T, Münch T, Scholz U, Mascher M. 2017. MISA-web: A web server for mi-
225 crosatellite prediction. *Bioinformatics* 2017, 33, 2583–2585
- 226 6. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence
227 data. *Bioinforma Oxf Engl*. 2014;30:2114–20.
- 228 7. Brandizzi F, Grilli Caiola M. 1996. Quantitative DNA analysis in different *Crocus* species
229 (Iridaceae) by mean of flow cytometry. *Giornale Bot. Italiano* 130: 643-645.
- 230 8. Brandizzi F, Grilli Caiola M. 1998. Flow cytometric analysis of nuclear DNA in *Crocus*
231 *sativus* and allies (Iridaceae). *Plant Syst. Evol*. 211:149-154.
- 232 9. Busconi M, Soffritti G, Stagnati L, Marocco L, Martínez JM, De Los Mozos Pascual M,
233 Fernandez JA. 2018. Epigenetic stability in Saffron (*Crocus sativus* L.) accessions during
234 four consecutive years of cultivation and vegetative propagation under open field conditions.
235 *Plant Sci.*, 277 (2018), pp. 1-10
- 236 10. Campbell MS, Holt C, Moore B, Yandell M. 2014. Genome annotation and curation using
237 MAKER and MAKER-P. *Curr Protoc Bioinforma*. 2014;48:4.11.1–39.
- 238 11. Chikhi, R.; Medvedev, P. 2014. Informed and automated k-mer size selection for genome
239 assembly. *Bioinformatics*, 30, 31–37.

12. Choi, J.Y, Lye, Z.N, Groen, S.C. *et al.* 2020. Nanopore sequencing-based genome assembly and evolutionary genomics of circum-basmati rice. *Genome Biol* 21, 21 (2020). <https://doi.org/10.1186/s13059-020-1938-2>
13. David M. Goodstein, Shengqiang Shu, Russell Howson, Rochak Neupane, Richard D. Hayes, Joni Fazo, Therese Mitros, William Dirks, Uffe Hellsten, Nicholas Putnam, and Daniel S. Rokhsar. 2012. Phytozome: a comparative platform for green plant genomics, *Nucleic Acids Res.* 40 (D1): D1178-D1186
14. Edwards, D, Batley, J. 2010. Plant genome sequencing: applications for plant improvement. *Plant Biotech. J.* 8: 2–9.
15. Feng S, He R, Lu J, Jiang M, Shen X, Jiang Y, Wang Z and Wang H. 2016. Development of SSR Markers and Assessment of Genetic Diversity in Medicinal *Chrysanthemum morifolium* Cultivars. *Front. Genet.* 7:113. doi: 10.3389/fgene.2016.00113
16. Hare EE, Johnston JS. 2011. Genome size determination using flow cytometry of propidium iodide-stained nuclei. *Methods Mol Biol.* 772:3-12. doi: 10.1007/978-1-61779-228-1_1. PMID: 22065429.
17. Harkess A, Zhou J, Xu C. *et al.* 2017. The asparagus genome sheds light on the origin and evolution of a young Y chromosome. *Nat Commun* 8, 1279 (2017). <https://doi.org/10.1038/s41467-017-01064-8>
18. Jain M, Srivastava P, Verma M. *et al.* 2016. *De novo* transcriptome assembly and comprehensive expression profiling in *Crocus sativus* to gain insights into apocarotenoid biosynthesis. *Sci Rep* 6, 22456. <https://doi.org/10.1038/srep22456>
19. Jia Ye, Yong Zhang, Huihai Cui, Jiawei Liu, Yuqing Wu, Yun Cheng, Huixing Xu, Xingxin Huang, Shengting Li, An Zhou, Xiuqing Zhang, Lars Bolund, Qiang Chen, Jian Wang, Huanming Yang, Lin Fang, Chunmei Shi. 2018. WEGO 2.0: a web tool for analyzing and plotting GO annotations, 2018 update, *Nucleic Acids Research*, Volume 46, Issue W1, 2 July 2018, Pages W71–W75, <https://doi.org/10.1093/nar/gky400>
20. Jinpu Jin, Feng Tian, De-Chang Yang, Yu-Qi Meng, Lei Kong, Jingchu Luo, Ge Gao. 2017. PlantTFDB 4.0: toward a central hub for transcription factors and regulatory interactions in plants, *Nucleic Acids Research*, Volume 45, Issue D1, January 2017, Pages D1040–D1045, <https://doi.org/10.1093/nar/gkw982>
21. Langmead, B. 2010. Aligning short sequencing reads with Bowtie. *Curr. Protoc. Bioinforma.* 32, 11.7.1–11.7.14.
22. Li H, Durbin R. 2010. Fast and accurate long-read alignment with burrows-wheeler transform. *Bioinformatics.* 26(5):589–95.

23. Liu, J, Seetharam, A.S, Chougule, K. *et al.* 2020. Gapless assembly of maize chromosomes using long-read technologies. *Genome Biol* 21, 121. <https://doi.org/10.1186/s13059-020-02029-9>
24. Luo, R.; Liu, B.; Xie, Y.; Li, Z.; Huang, W.; Yuan, J.; Wang, J. 2012. SOAPdenovo2: An empirically improved memory-efficient short-read de novo assembler. *Gigascience* 2012, 1, 18.
25. Maggi MA, Bisti S and Picco C. 2020. Saffron: Chemical Composition and Neuroprotective Activity, *Molecules* 2020, 25, 5618. doi:10.3390/molecules25235618.
26. Magotra S, Bhagat N, Ambardar S. *et al.* 2021. Field evaluation of PGP *Bacillus* sp. strain D5 native to *Crocus sativus*, in traditional and non traditional areas, and mining of PGP genes from its genome. *Sci Rep* 11, 5454. <https://doi.org/10.1038/s41598-021-84585-z>.
27. Marçais, G, and Kingsford, C. 2011. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics* 27, 764–770. doi: 10.1093/bioinformatics/btr011
28. Mir MA, Mansoor S, Sugapriya M, Alyemeni MN, Wijaya L, Ahmad P. 2021. Deciphering genetic diversity analysis of saffron (*Crocus sativus* L.) using RAPD and ISSR markers. *Saudi Journal of Biological Sciences*. 28(2) : 1308-1317
29. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res.* 2007 Jul;35(Web Server issue):W182-5. doi: 10.1093/nar/gkm321. PMID: 17526522; PMCID: PMC1933193.
30. Mzabri I, Addi M, Berrichi A. 2019. Traditional and Modern Uses of Saffron (*Crocus Sativus*). *Cosmetics*. 6(4):63. <https://doi.org/10.3390/cosmetics6040063>
31. Nemati Z, Harpke D, Gemicioglu A, Kerndorff H, Blattner F.R. 2019. Saffron (*Crocus sativus*) is an autotriploid that evolved in Attica (Greece) from wild *Crocus cartwrightianus*. *Mol Phylogenet Evol*, 136, pp. 14-20
32. Ranallo-Benavidez TR, Jaron KS, Schatz MC. 2020. GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. *Nat Commun.* 11(1):1432. Published 2020 Mar 18. doi:10.1038/s41467-020-14998-3
33. Rogers S. O. and Bendich A. J. 1994. Extraction of total cellular DNA from plants, algae and fungi. In *Plant molecular biology manual*. (ed. S. B. Gelvin and R. A. Schilperoort), 2nd edition, vol. D1, pp. 1–8. Kluwer Academic Press, Dordrecht.
34. Rubio-Moraga A, Castillo-López R, Gómez-Gómez R, Ahrazem O. 2009. Saffron is a monomorphic species as revealed by RAPD, ISSR and microsatellite analyses. *BMC Res. Notes*, 2 (1), p. 189, 10.1186/1756-0500-2-189

35. Samarghandian S, Borji A. 2014. Anticarcinogenic effect of saffron (*Crocus sativus* L.) and its ingredients. *Pharmacogn. Res.* 6:99–107. doi: 10.4103/0974-8490.128963.
36. Simão, F.A.; Waterhouse, R.M.; Ioannidis, P.; Kriventseva, E.V.; Zdobnov, E.M. 2015. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 2015, 31, 3210–3212.
37. Siracusa, L, Gresta, F, Avola, G, Albertini, E, Raggi, L, Marconi, G, et al. 2013. Agronomic, chemical and genetic variability of saffron (*Crocus sativus* L.) of different origin by LC-UV-vis-DAD and AFLP analyses. *Genet. Resour. Crop Evol.* 60, 711–721. doi: 10.1007/s10722-012-9868-9
38. Smit, A.F.A.; Hubley, R.; Green, P. 2010. RepeatMasker Open-4.0.. Available online: <http://www.repeatmasker.org>
39. Wang Z, Hobson N, Galindo L, et al. 2012. The genome of flax (*Linum usitatissimum*. L) assembled de novo from short shotgun sequence reads. *Plant J.* 72(3):461–473.
40. Xia E, Tong W, Hou Y, An Y, Chen L, Wu Q, Liu Y, Yu J, Li F, Li R, et al. 2020. The reference genome of tea plant and resequencing of 81 diverse accessions provide insights into genome evolution and adaptation of tea plants. *Mol Plant.* 13(7):1013–1026.
41. Xu L, Dong Z, Fang L, Luo Y, Wei Z, Guo H, Zhang G, Gu YQ, Coleman-Derr D, Xia Q, Wang Y. 2019. OrthoVenn2: a web server for whole-genome comparison and annotation of orthologous clusters across multiple species. *Nucleic Acids Res.* 2019 Jul 2;47(W1):W52–W58. doi: 10.1093/nar/gkz333. PMID: 31053848; PMCID: PMC6602458.
42. Zhang YH, Zheng LL, Zheng Y, Zhou C, Huang P, Xiao X, et al. 2019. Assembly and annotation of a draft genome of the medicinal plant *Polygonum cuspidatum*. *Front Plant Sci.* 10:1274. <https://doi.org/10.3389/fpls.2019.01274>.
43. Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. *Bioinformatics.* 2013; 29(21):2669–77.

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345 **Table 6:** Number of genes annotated against NR and Viridiplantaea database depicting more num-
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350 processes.

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355 **Fig 4a:** Orthology analysis of *Crocus sativus* with neighbouring plants from same order along with
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Libraries insert Size	Sample Name	% GC	Sequences (Million Read)	Data (in Giga- bases)
800	Lib1_800_R1	46%	93.2	13.98
	Lib1_800_R2	46%	93.2	13.98
	Lib2_800_R1	46%	357.8	53.68
	Lib2_800_R2	46%	357.8	53.68
500	Lib3_500_R1	46%	300.1	45.02
	Lib3_500_R2	46%	300.1	45.02
	Lib4_500_R1	46%	320.0	48.01
	Lib4_500_R2	46%	320.0	48.01
Total			2142.2	321.36

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374 **Table 2:** Assembly statistics of *C. sativus* genome using soapdenovo2 and MaSuRCA *de-novo* as-
375 semblers.

	Soapdenovo2			MaSuRCA
Assemblies	-	Cs_Assembly_1	-	Cs_Assem- bly_2
kmers	69	71	73	99
N50 Scaffold (bases)	1443	1596	1508	1863
Number of Scaffolds	1537310	1505129	1433675	2564042
Largest Scaffold (bases)	45973	45973	43370	46734
Total sequence length	2684437407	2787926280	2589039086	3014612563
GC%	43.2	43.2	43.2	43.2
Genome Coverage (%)	75.01%	77.90%	72.34%	84.24%
BUSCO (%)	7.32%	7.81%	7.05%	44.46%

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378 **Table 3:** Mapping WGS raw reads and previous published transcriptome data to Cs_assembly_2.

Data Type	WGS raw reads in present study	<i>C.sativus</i> transcripts (Jain et al., 2016)	<i>C.sativus</i> transcriptome raw reads (Baba et al 2015)
Source of Data	Present study	Jain et al., 2016	Baba et al 2015
Total number of reads/transcripts	2135957246	327920	59043670
Mapped reads/transcripts	1864292472	327643	54330850
Mapping Percentage	87.28 %	99.92 %	92.02 %

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382 **Table 4:** Classification of repetitive sequences in *C. sativus* genome representing abundance of
383 Simple repeats and LTRs.

Repetitive region	Numbers
Simple repeats	415561
LTR	260472
Low_complexity	64624
DNA	64205
LINE	45739
Satellite	3946
RC_Helitron	3072
rRNA	2749
SINE	848
tRNA	650
Other	340
snRNA	54
Retroposon	15
Total	862275

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388 **Table 5:** SSR markers from *Crocus sativus* draft genome (Cs_assembly_2) depicting the more rela-
389 tive abundance of monomeric repeat microsatellite.

SSR types	count	relative %age	Most abundant	%age
monomeric repeat microsatellite	486140	50.4%	“A”	44.6 %
dinucleotide repeat microsatellite	294819	30.5%	“TA”	16.5 %
trinucleotide repeat microsatellite	146991	15.2%	“TTG”	5.72 %
Tetranucleotide repeat microsatellite	15375	1.59%	“AAAT”	7.7 %
Pentanucleotide repeat microsatellite	8596	0.9%	“TATAT”	3.2 %
Hexanucleotide repeat microsatellite	12310	1.27%	“TAACCC”	5.3 %
Total	964231			

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393 ber of proteins annotated against Viridiplantaea database.

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Databases	Total maker annotated	Total annotated proteins	>40% percentage identity & > 70% query coverage	Unique accession numbers
Viridiplantaea	254038	146118	107553	52546
NR	254038	143745	107385	52436

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403 Figures:

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de novo genome assembly pipeline

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