小麦分蘖形态学特征 X 射线-CT 无损检测

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摘 要:针对传统小麦分蘖形态学特征需采用人工接触或有损方法获取,不仅过程繁琐、主观性强而且会影响小麦后续 生长。为实现小麦分蘖性状快速、准确、无损测量,该文提出一种基于 X 射线断层成像的小麦分蘖形态学特征提取方法。 首先构建了小麦分蘖 X-ray CT 断层扫描成像系统,采用滤波反投影(filtered back projection, FBP)断层重建算法和图形 处理单元(graphics processing unit, GPU)加速算法快速获取小麦分蘖茎秆断层图像,设计了专门的分蘖图像分析算法实 现对小麦分蘖形态学特征参数(分蘖数、分蘖角度、分蘖茎粗和分蘖壁厚)的无损检测。该研究对 107 株小麦植株测量 结果表明:分蘖数测量准确率可达 100%,分蘖角度、茎粗和壁厚的平均测量误差分别为 3.65%, 4.84%和 7.86%。该技 术相较于人工测量方法和石蜡切片方法,能够对小麦分蘖形态学特征进行较为精准无损检测,实现单株测量效率约 200 s, 对于小麦功能基因组和抗倒伏能力品种的筛选具有重要的研究意义。

关键词:X射线;断层重建;图像处理;小麦分蘖;无损检测

doi: 10.11975/j.issn.1002-6819.2017.14.027

中图分类号: S512; S123 文献标志码: A

文章编号: 1002-6819(2017)-14-0196-06

吴 迪,杨万能,牛智有,黄成龙.小麦分蘖形态学特征 X 射线-CT 无损检测[J].农业工程学报,2017,33(14):196-201. doi: 10.11975/j.issn.1002-6819.2017.14.027 http://www.tcsae.org

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0 引 言

小麦是中国乃至世界最主要的粮食作物之一^[1-3]。小 麦是人类淀粉的主要摄取来源,同时富含蛋白质、脂肪、 矿物质等丰富的营养^[4-5]。为进一步提高小麦产量,小麦形 态改良^[6]逐渐从矮化育种转向增加株高方向进行育种^[7-8]。

小麦分蘖起到了养分输送与抗倒伏的作用,分蘖性状与 产量密切相关^[9],小麦分蘖数与产量直接相关,分蘖角度 影响小麦的株型结构,分蘖茎粗、壁厚决定小麦的抗倒 伏能力^[10-11],因此小麦分蘖性状研究对小麦育种具研究具 有十分重要的意义^[12]。

随着小麦功能基因组及分子育种的飞速发展,需要 对大量品种进行表型鉴定。传统的小麦分蘖检测手段主 要依靠人工完成,通过计量工具测量并计算水稻分蘖的 分蘖数量、分蘖角度、分蘖茎粗、分蘖壁厚^[13-14]。人工 检测为有损离体测量,具有测量精度低、可重复性差等 缺点,已经远远不能满足当前小麦育种的需求。因此研 发一种无损且精度高的小麦分蘖性状提取技术,是小麦 功能基因组发展急需解决的一个问题^[15-16]。

近年来,机器视觉技术因其无损性和高效性正广泛 应用于农产品检测^[17-18]。杜光源等^[19]利用核磁共振可以 测量小麦叶片衰老态势,邓继忠等^[20]采用图像识别技术 可以对小麦腥黑穗病进行特征提取与分类,张云鹤等^[21] 利用可见光视觉技术研发了作物分蘖直径变化测量仪。 Bauriegel 等^[22] 采用高光谱成像技术可以对小麦镰刀霉病 进行早期预测。综上所述,基于可见光、高光谱、核磁 共振等反射成像技术,只能获取对小麦表观性状的信息, 无法用于获取分蘖内结构信息。而 X 射线作为一种透射 成像技术,目前已经应用于土壤孔隙检测^[23]、植物根系 研究^[24],木材内部裂纹、孔洞缺陷检测^[25],水稻分蘖数 测量^[26],为小麦分蘖性状获取提供了一种可行的途径。

本文通过采用自动控制系统、微型 CT (computerized tomography) 技术及图像处理技术相结合的方式,研制了一套自动化的表型提取设备,以完成对小麦分蘖数、分蘖角度、分蘖茎粗、分蘖壁厚等形态学特征性状的在体无损检测。

1 小麦 CT 系统组成和成像结构

1.1 系统组成

本文设计的 CT 系统主要由计算机 1 (双核 CPU 3.2 GHz,内存 2.98 GB,显卡 ATI Radeon HD 5450)、PLC 控制器 2 (programmable logic controller)(CP1H,OMRON,日本)、微焦斑射线源 3 (Nova 600,OXFORD,英国)、旋转平台 4、平板探测器 5 (2520DX,VARIAN,美国)等 5 个功能模块组成^[27-28],系统结构示意图如图 1 所示。先将待测样本小麦放置在载物旋转台上,微焦斑射线源发射 X 射线穿透小麦样本,通过计算机系统发送指令给 PLC,PLC 控制载物旋转台实现样本的等间距旋转,利用平板探测器获取每个角度下的 X 射线吸收面阵列图像,取同一高度的全部角度图像组成新的图像,即投影正弦图,再结合滤波反投影 FBP (filtered back

收稿日期: 2017-02-26 修订日期: 2017-06-20

基金项目:国家高新技术发展计划(863 计划,2013AA102403);国家自然科学基金项目(31600287);湖北省科研条件与资源研究开发(2015BCE044)作者简介:吴迪,博士生,主要从事农业信息无损检测研究。武汉 华中农业大学工学院,430070。Email: 380524590@qq.com

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projection)算法^[29]和图形处理 GPU (graphics processing unit)加速算法^[30]得到断层重建图像,最后基于图像处理 技术对断层重建图像进行图像分析与处理,测算并提取 出小麦分蘖的性状参数^[31]。CT 重建的原理源于 X 射线通 过物体时衰减的物理规律^[32-33],即郎伯定律如式 (1), 然后结合 Radon 变换得到投影重建图像。

$$I = I_0 \mathrm{e}^{-\mu L} \tag{1}$$

式中 I 为平板探测器探测到的 X 射线的强度, I_0 为入射 X 射线的强度 (Ci), μ 为成像物质的吸收系数, L 为物体的 厚度 (mm)。



a. 计异仇反径而平元 b. 小友分蘖X别线成像装置 a. Computer and control unit b. X-ray imaging device for wheat tiller

1. 计算机 2. PLC 控制器 3. 微焦斑射线源 4. 旋转平台 5. 平板探测器 6. 小麦试验样本

1. Computer 2. PLC controller 3. Micro focus X-ray source 4. Rotation stage 5. Flat panel detector 6. Wheat sample

Fig.1 Schematic diagram of CT system

本试验的研发主要是基于美国国家仪器公司(National Instruments)的 LabVIEW 软件、VARIAN 公司的 VIVA 软件、美国微软公司的 Microsoft Visual Studio 软件。首 先通过 VIVA 软件完成平板探测器的偏移校准和增益校 准。然后通过 LabVIEW 软件完成 X 射线图像采集和载物 台旋转控制功能。再利用 Microsoft Visual Studio 软件来 实现断层重建和分蘖图像处理算法,并生成动态链接库 (dynamic link library, DLL)。最后利用 LabVIEW 将上述 功能集成,实现小麦分蘖图像校准、采集、处理一体化。

1.2 成像结构

由于微焦斑射线源的出射角为 33°,结合平板探测器的尺寸(195 mm×244 mm),可计算出当射线源与平板 探测器之间的距离为 634 mm 时射线源发出的 X 射线刚 好全部覆盖平板探测器,考虑到小麦样本的实际尺寸并 获取尽可能高的空间分辨率,本试验中选取的微焦斑射 线源至试验样本的距离为 305 mm,基于该成像结构可获 取的 X-ray 投影及重建分辨率可达 61 µm×61 µm。试验 选用的具体性能参数如表 1 所示。

	表 1 小麦 CT 系统性能参数
Tabla 1	CT system performance parameters for wheat

参数 Parameters	性能参数值 Performance parameter value
X射线阳极电压 X-ray anode voltage/kV	40
X 射线阳极功率 X-ray anode power/W	40
投影图片分辨率 Projection image resolution/(µm×µm)	61×61
断层重建图片分辨率 Tomographic reconstruction image resolution/(µm×µm)	61 × 61
小麦与探测器间的距离 Distance between wheat and detector/mm	305
X 射线源与探测器间的距离 Distance between X-ray source and detector/mm	634
每株小麦采集的图片数量 Number of image collected per wheat	360
单次旋转角度 Single rotation angle/(°)	1
像素面积 Pixel area/(mm×mm)	195×244
像素矩阵 Pixel matrix	1536×1920
像素间距 Pixel pitch/(µm×µm)	127×127

2 试验材料及方法

2.1 试验材料

本试验一共选取 5 个不同的小麦品种(华麦 2668, 华麦 2533,华麦 166,华麦 2566,华麦 2153),每个品 种有 25 株,一共 125 株小麦样本,试验样本盆栽种植。 用于种植试验样本小麦的土壤先在太阳下晒干,然后将 晒干的土壤初步碾碎,通过 8 mm 的网筛去除土壤中的石 头和杂草,保证土壤的均一性,最后将氮、磷、钾肥料 按照 2:1:1 的配比搅拌均匀。在小麦生长过程中提供 正常的水肥管理,待小麦播种 90 d 后开始进行小麦分蘖 形态参数的无损检测及人工验证。

2.2 试验具体步骤

CT 系统具体的控制操作步骤如下: 1) 打开 X 射线 源冷却装置,使得射线源温度控制在 20 ℃以内; 2) 打 开射线源和探测器,对系统进行校准; 3) 探测器采集图 片,并判断采集的图片信噪比是否符合要求; 4) 采集完 一株样本后,放置下一株样本继续采集,直至全部样本 采集完成; 5)关闭射线源、探测器和计算机。

由于天气等外在原因,最终用于试验的小麦样本为 107 株小麦。人工测量的具体测量步骤为:1)2 名试验 人员分别对每株小麦数取分蘖数量,用量角器分别获取 分蘖角度,取2 人所得值的平均值做为分蘖数和分蘖角 度的人工测量值;2)对每株小麦进行 CT系统检测,采 集投影图片;3)将每株小麦剪剩至1根分蘖为止(方便 后期与人工值进行匹配对应),对其再次进行 CT系统 检测,采集投影图片;4)2名试验人员利用游标卡尺, 分别对剩下的1根分蘖测量分蘖茎粗和分蘖壁厚,取2 人所得值的平均值做为分蘖茎粗和分蘖壁厚的人工测 量值。

2.3 试验方法和性状提取

本文提出的基于 X 射线微型 CT 的小麦分蘖性状无 损提取方法,技术路线如图 2 所示,具体效果如图 3 所 示,具体内容主要包括:



注: ROI 为感兴趣区域; FBP 为滤波反投影算法; GPU 为图像处理单元。 Note: ROI is the region of interest; FBP is the filtered back projection algorithm; GPU is graphic processing unit.

图 2 小麦分蘖性状提取技术路线

Fig.2 Flow chart of technical route for wheat tiller traits extraction

图1 CT系统组成示意图



注: *α*为分蘖角度, (°); *x* 为平面内两点的欧式距离, mm; *y* 为两平面间的 距离, mm; *D* 为分蘖茎粗, mm; *a* 为分蘖长轴长度, mm; *b* 为分蘖短轴 长度, mm; *d* 为分蘖壁厚, mm。

Note: α is the tiller angle, (°); x is the Euclidian distance between two point in the same plane, mm; y is the distance between two planes, mm; D is the tiller diameter, mm; a is the major axis of wheat tiller, mm; b is the minor axis of wheat tiller, mm; d₁ is the tiller cavity diameter, mm; d is the tiller wall thickness, mm.

图 3 图像处理及分蘖性状提取流程

Fig.3 Flow chart of image processing and tiller traits extraction

2.3.1 小麦分蘖断层结构快速重建

对于小麦植株如图 3a, 首先利用 CT 系统获取小麦 分蘖 X-ray 投影图像如图 3b, 然后选取两个不同高度及 360 个角度下的投影图像组成正弦图如图 3c,再基于滤波 反投影 FBP 算法,并结合平板探测器的偏移校准、增益 校准降噪方法来获取清晰的小麦分蘖断层结构,其重建 效果如图 3d 所示。

本研究还利用感兴趣区域(region of interest, ROI) 算法和图像处理单元加速算法,进一步减少 CT 断层重建 所消耗的时间,实现小麦分蘖内部断层结构的快速重建。 2.3.2 小麦分蘖内部性状的提取

小麦分蘖断层图像处理及性状提取步骤如下所述。

针对小麦分蘖断层重建图像,首先采用大津算法 (OTSU)自动计算分割阈值,利用该阈值将图像进行二 值化^[32](如图 3e),然后利用去除小区域算法消除背景噪 声(如图 3f),再采用连通区域标记方法识别出单个小麦 分蘖区域(如图 3g),最后计算出小麦分蘖性状的分蘖数、 分蘖茎粗、分蘖壁厚和分蘖角度(如图 3h),具体计算方法 如下。

分蘖数量的计算:数取连通区域的个数;

分蘖角度α的计算:对同一对象,分别在两个不同高度的断层面取质心,投影到同一平面后,计算平面内 2 点的欧氏距离 x,并且系统已知两个断层面的垂直距离 y, 因此可以计算出分蘖角度α(°)。

$$\alpha = \arctan(x/v) \tag{2}$$

分蘖茎粗 D 的计算:将单个分蘖先进行旋转,再测量长轴 a 与短轴 b,从而计算出茎粗

$$D = (a+b)/2 \tag{3}$$

分蘖壁厚 *d* 的计算:按茎粗的计算方法先获取内部 髓腔的直径 *d*₁,从而计算出壁厚

$$d = (D - d_1)/2 \tag{4}$$

2.3.3 系统评估

基于上述图像分析过程,将系统测量的 107 份小麦 植株分蘖数、分蘖角度、分蘖茎粗、分蘖壁厚与人工测 量值对比来评价该方法的精度。测量结果所得的平均绝 对百分比误差(mean absolute percentage error, MAPE) 和标准差(root mean square error, RMSE)的计算方法为

MAPE =
$$\frac{1}{n} \sum_{i=1}^{n} \frac{|x_{ai} - x_{mi}|}{x_{mi}} \times 100\%$$
 (5)

RMSE =
$$\sqrt{\frac{1}{n} \sum_{i=1}^{n} (x_{ai} - x_{mi})^2}$$
 (6)

式中n为样本数,xai为系统测量值,xmi为人工测量值。

3 试验结果与分析

3.1 系统测量精度

基于上述小麦分蘖重建和图像分析过程,本试验对 107份小麦植株提取的所有分蘖内部性状进行分析。将本 系统测量的分蘖数、分蘖角度、茎粗和壁厚与人工测量 值对比来评价本系统的精度。其中,人工测量小麦样本 的植株位置与系统测量高度基本一致(均为距离土面 5 cm 处),以减少不同高度下所产生的分蘖角度、茎粗和 壁厚误差。

图 4a 是分蘖数的系统测量值与人工测量值的对比散 点图,可看出 R^2 =1,MAPE = 0%,RMSE = 0,本系统 测量分蘖数的准确性为 100%。图 4b 是分蘖角度的系统 测量值与人工测量值的对比散点图,可看出 R^2 = 0.77, MAPE = 3.65%,RMSE = 2.96°。图 4c 是茎粗的系统测量 值与人工测量值的对比散点图,可看出 R^2 = 0.91,MAPE = 4.84%,RMSE = 0.17 mm,散点大部分落 1:1 线右侧, 说明大部分系统测量值较人工测量大,这可能是由于试 验人员用游标卡尺对分蘖茎粗进行测量时,卡尺用力将 茎秆夹紧时,茎秆变细导致的人工测量误差。图 4d 是 壁厚的系统测量值与人工测量值的对比散点图,可看出 R^2 = 0.87,MAPE = 7.86%,RMSE = 0.12 mm。结果表 明本系统具有较高的测量精度,系统测量值与人工测量 值具有较好的一致性,本研究结果说明基于 X-ray CT 技 术可以准确的测量小麦分蘖形态学特征。

3.2 系统测量效率

本套系统的图像采集时间大约 200 s/株,图像重建、 图像分析和性状提取时间大约 120 s/株,由于图像重建、 图像分析和性状提取可以与图像采集并行执行,故只需 要考虑二者最长的时间,即本系统的测量效率为 200 s/株。 如该系统一天连续工作 24 h,理论上 1 d 可以测量 432 株 小麦。 人工测量效率会受到分蘖复杂程度的影响,分蘖越 复杂,人工测量效率越慢,可重复性差,容易产生测量 误差,但系统不会受到分蘖复杂程度的影响,能准确的 测量小麦分蘖形态学特征。



图 4 系统测量与人工测量结果

Fig.4 Results of system measurement and manual measurement

3.3 系统对比分析

本研究系统与其它测量方法的对比结果如表 2 所示。 本方法与人工测量方法相比,首先可以实现无损表型性 状的获取,其次在测量效率上人工处理该 107 株小麦植 株花费时间大约为 30 h,平均每株测量效率约为 1 000 s,而本系统大约只需要 6 h,测量效率提高约 5 倍, 在测量精度上本系统可以与人工测量相比具有较高的一 致性,并能克服人工测量上存在主观、易疲劳的缺点。 与石蜡切片法相比,同样可以克服有损的缺点并与其报 道的测量效率相比提高约 9 倍^[34]。与基于多光谱的可见 光预测方法相比,本方法不但可以获取小麦分蘖的内部 信息如分蘖壁厚,还能克服小麦分蘖之间的交叠提供更 精准的小麦分蘖性状,以分蘖数测量为例可见光预测方 法能达到的最高决定系数为 0.85^[35],而本系统可达到 1。

表 2	该系统与其它测量方法的性能对比	

Table 2 Performance comparison between CT system and other methods

方法 Methods	表型性状 Phenotypic traits	是否有损 Destructive or not	测量效率 Measuring efficiency/ (s·株 ⁻¹)
人工测量	分蘖数量,分蘖茎粗, 分蘖壁厚,分蘖角度	是	1 000
CT 系统测量	分蘖数量,分蘖茎粗, 分蘖壁厚,分蘖角度	否	200
石蜡切片法	分蘖茎粗,分蘖壁厚, 维管束数量和面积	是	1 800
可见光预测	分蘖数	否	快速

4 结论与讨论

小麦分蘖为小麦生长提供营养输送作用,其茎粗、 壁厚与抗倒伏能力密切相关,分蘖数、分蘖角度直接影 响小麦的株型,小麦分蘖形态学特征性状研究对小麦遗 传育种及功能基因研究具有要用意义,本研究提出了一 种基于 X-CT 测量小麦分蘖形态的方法。

1)本文针对小麦分蘖形态学特征性状获取,构建了 一套 X 射线断层成像系统,通过该系统可以无损、快速 获取小麦茎秆的断层图像,最后采用图像分析算法实现 对小麦分蘖形态学特征的在体、无损、自动检测。针对 107 株小麦植株进行分蘖断层重建,自动获取分蘖性状参 数包括分蘖数、分蘖角度、茎粗和壁厚,试验结果显示 测量精度(mean absolute percentage error, MAPE)分别 为100%、96.35%、95.16%、92.14%;标准差(root mean square error, RMSE)分别为0、2.96°、0.17 mm、0.12 mm。 以上结论表明本套系统具有较高的测量精度。

2)本套系统方便扩展应用于水稻、玉米、油菜等其 他作物,该技术可与现有植物表型提取技术(可见光、 近红外、红外成像等)相集成,为植物表型性状获取提 供一种新的技术手段。

3)传统的测量小麦分蘖形态学特征主要有可见光检测、人工有损检测和石蜡切片电镜检测。其中可见光只能获取作物的表面信息,分蘖之间的遮挡对测量精度影响较大,且无法测量壁厚,后两种方法虽然可以实现本研究目标,但均为有损检测,不仅效率低下且无法重复。因此本研究为小麦分蘖形态测量提供了一种新的方法。

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Non-destructive detection of wheat tiller morphological traits based on X-ray CT technology

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Abstract: Wheat tillers play an important role for nutrition transport to support the wheat growth. The wheat stem diameter and thickness are closely related to lodging resistance. Meanwhile tiller number and tiller angle directly determine the plant type of wheat. Therefore, the morphological trait extraction of wheat tillers is very important to the study of wheat genetics research, breeding improvement and functional genes location. With the development of the wheat cultivation and genetic breeding, the fast and accurate measurement of morphological traits for wheat tillers is imperative. However, the traditional method for tiller trait measurement is still manual, which is destructive and time consuming. Although a lot of efforts had been made to extract the tillers traits generally based on visible light, it is not able to acquire the inner information of wheat tiller and is affected seriously by tillers overlap. To solve the problem, a nondestructive technology for wheat tillers measurement was proposed and equipped with X-ray CT imaging device. In this study, the X-ray CT imaging system was constructed with the Micro-focus X-ray source and flat detector, which was used to obtain the sinogram images of wheat tiller with the spatial resolution 61 µm by 61 µm, and totally 360 images were collected for every one degree rotation for each plant. Then the FBP and GPU algorithms were adopted to reconstruct the tomography image of wheat tillers based on the sinogram images, and the inner information of wheat tiller was visible in the image. Moreover, the specialized image analysis algorithms were designed to analyze the wheat tomography image, in which the algorithms of background subtraction, OTSU segmentation, removing small region, and connected region identification were applied to extract the tiller regions. After that, the wheat tiller morphological traits were extracted by the following methods, the tiller numbers were counted based on the number of connected areas, the stem diameter was computed by the information of area external rectangle, the tiller wall thickness was extracted with the information of area external rectangle and cavity rectangle, and tiller angle was obtained by the triangle relation of tomography images at different heights. Finally this method was evaluated by 107 wheat plants, which belonged to five different wheat varieties. After the wheat plants were measured by the system automatically, the plants were measured by manual method for comparison to evaluate the system measurement accuracy. The experimental results showed that the system measurement accuracy of the tiller number was 100%, the mean absolute percentage error of tiller angle, the stem diameter and the stem wall thickness were 3.65%, 4.84% and 7.86%, respectively and the RMSE for above traits were 2.96, 0.17 mm, 0.12 mm, respectively. The R2 value of tiller angle, the stem diameter and the stem wall thickness were 0.77, 0.91 and 0.87, respectively. The results demonstrated that this method had a good consistency with manual method, and performed a high accuracy for wheat tiller morphological trait measurements. In this study, the image acquisition efficiency was about 200 s per plant and the time used for image analysis was about 120 s per plant. Considering the parallel implement of image acquisition and analysis, the system efficiency was about 200 s per plant and was able to measure approximate 432 wheat plants in one day. Compared with manual method, this technology was able to detect the internal information of wheat tiller with high-accuracy and nondestructive. Moreover, it was able to extract novel phenotypic traits, which may contribute to the functional genomics and lodging resistance research of wheat plants. In future, more detailed information of wheat tiller such as vascular bundle, leaf sheath could be analyzed based on the higher resolution X-ray imaging device and more intelligent algorithms. **Keywords:** X rays; computerized tomography; image processing; wheat tiller; nondestructive testing